

Abstract

POWELL, BRADFORD EDMUND. Interactions between the ants *Linepithema humile*, *Tapinoma sessile* and aphid mutualists. (Under the direction of Jules Silverman.)

Invasive species have major impacts on the ecosystems they invade. Among the most disruptive groups of invasive species are ants. Invasive ants have caused losses in biodiversity among a wide range of taxa, including birds, mammals, lizards, but especially towards ground nesting arthropods such as native ants. Why native ants are so susceptible to invasion and how invasive ants are able to sustain massive population growth remain unclear. It has been suggested that invasive ants utilize carbohydrate resources from hemipteran exudates to fuel aggressive foraging and colony expansion. Perhaps invasive ants are simply more proficient at usurping these resources, maintaining higher hemipteran populations, etc.?

I use a model invasive, the Argentine ant (*Linepithema humile*) and a native ant (*Tapinoma sessile*) to compare hemipteran tending ability and ant competition. Through a series of laboratory and field experiments I evaluated 1) sucrose consumption by individual and groups of ant workers, 2) the effect either ant species had on hemipteran population growth rates in a predator-free space, 3) the defensive ability of either ant against hemipteran predators and parasitoids, and 4) the proportion of invasive ants required to displace a native colony from a hemipteran resource. Whereas there was no difference between the species in their ability to sequester liquid resources, recruitment strategies differed considerably. Hemipteran populations in the presence of *L. humile* grew larger in a predator free environment and populations exposed to predators were better defended by *L. humile* than *T. sessile*. *L. humile* was able to displace *T. sessile* from a nest site even when workers were

outnumbered two to one. Aggression by either ant species was significantly reduced without hemipterans present. By having intrinsic effects on hemipteran population growth rate and being better defenders, invasive ants can sustain larger carbohydrate resources that can partially explain their rapid growth and spread.

Interactions between the ants *Linepithema humile*, *Tapinoma sessile* and aphid mutualists

by
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Dedication

I dedicate this work to my loving wife,
who has put up with my impoverished wages for the better part of a decade

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Literature review and overview of chapters

Forward/Note to reader:

The purpose of this review is to introduce the reader to two major groups of insects: aphids and ants. It will discuss how these groups interact with each other, followed by a discussion of the importance of invasive species. Then it will focus on Argentine ant (*Linepithema humile*) invasions and how they affect their introduced environments. Particular emphasis will be given to the ant's manipulation of hemipteran resources. The introduction will then transition into a proposal to quantify the invasive ability of Argentine ants vs. a model native, the odorous house ant (*Tapinoma sessile*).

Aphid background:

The aphids (Hemiptera: Aphididae) are one of the largest groups formerly in the Homoptera suborder, containing over 4000 species (Stadler & Dixon, 2005); however, the paraphyletic group is no longer taxonomically valid, and now is under the Sternorrhyncha suborder. These soft-bodied insects undergo one of the most complex forms of reproduction of all insects. Most of the aphids use apomictic parthenogenic reproduction, in which there is no meiotic cell division and the offspring are genetically identical to the mother. This form of reproduction occurs throughout the spring and summer, until cued by a seasonal shift in the photo/scotophase of the forthcoming winter (Nunes & Hardie, 1992; Hardie & Lees, 1983).

Once the photophase has reached a certain point, aphids will then begin to produce male and female alate sexuals. After nuptial flights, females will lay eggs that overwinter until the following spring (Borrer et al., 1989). In addition to their complex reproduction and ployphenism, aphids have a rather unique system of nutrient acquisition. They use their specialized mouthparts (stylets) to tap into the phloem of plants to acquire amino acids, sugars and other necessary nutrients for growth. The problem with plant phloem, however, is that certain nutrients (e.g. nitrogen) are very sparse and a large amount phloem must be processed in order to meet the aphid's needs (Chapman, 1998; Ponsen, 1990; Ponsen, 1987). To handle this large amount of liquid material aphids as well as other hemipterans have evolved a filter chamber (Ponsen, 1990; Ponsen, 1987; Rhodes et al., 1997) as well as the ability to excrete excess sugars and other waste material in the form of honeydew (Borrer et al., 1989; Chapman, 1998; Rhodes et al., 1997). The importance of the sugary excreta to other insects will be discussed later. For the aphid, honeydew excretion allows the insect to get rid of excess sugar and water and regulate osmolality. This excretory process is very uncommon in the rest of the Insecta, since most terrestrial insects have a limited supply of water and sugar from their food sources.

Aphids have to remain stationary for long periods of time while they feed on this abundant but nutritionally poor food source, and therefore aphid populations are

largely affected by external sources. Aphid clonal reproductive rate is contingent on a variety of external top down and bottom up stimuli. Bottom up effects mostly deal with plant quality (Addicott, 1978; Gruber & Dixon, 1988; Morris, 1992; Edson, 1985), but there is also evidence that soil biota can play a role in aphid performance (Belnap et al., 2005). While water and nutrients can certainly play a role in aphid population dynamics, top down effects can drastically change an aphid's reproductive potential. Climate, predation, inter and intraspecific competition are among the major external factors that influence aphids as well as most other organisms. However, among insects, ant herding or tending is not a common mutualistic and it is mostly restricted to the Sternorrhyncha, some groups of the Auchenorrhyncha (Insecta: Hemiptera), and the Lycaenidae larvae of the Lepidoptera, this review focuses on aphid tending. The details of ant-tending are discussed in detail later.

Ant Background:

The other major group in this study is the ants (Hymenoptera: Formicidae). Ants are arguably the most successful group of insects in terms of ecological breadth and sheer biomass. Nearly all insects interact in some way with the approximately 2 billion pounds of ants comprised of over 8,000 species in their lifetime (Hölldobler & Wilson, 1990). The ants also have a rather interesting reproductive scheme and it is in this marvel of evolution that the ants owe much of their success. Ants are known as eusocial or truly social organisms, which entails a list of specialized behaviors and

morphologies. The list includes colonial (group) living, a caste system, reproductive dominance, cooperative brood care and overlapping of generations; all of these characteristics are needed to be deemed a eusocial insect. Ants live in colonies of individuals ranging from only a couple hundred like the bullet ants (e.g. *Paraponera clavata*) of South America to several million as seen in Argentine ant (*Linepithema humile*) supercolonies around the world. The caste system in ants is made up of usually only two castes: reproductives and workers; however, some tropical species (e.g. *Eciton* or *Pheidole* spp.) have soldiers, much like termites. Due to their morphology, soldier ants are incapable of feeding themselves and rely on their worker sisters for all nutrition. The reproductive caste is restricted to usually only one or a few individuals that arguably maintain their control over the much larger worker caste through the use of pheromonal communication. The primer pheromones restrict ovarian development of the workers and provide information concerning task allocation, e.g. nursing and foraging. Defense of the colony is mainly accomplished through releaser pheromones. The remaining caste, the vast majority of individuals in a colony, is comprised of sterile workers that provide nourishment, brood care, nest construction and in most cases, defense of the colony. The workers provide care for their siblings over multiple generations (Borror et al., 1989; Chapman, 1998; Hölldobler & Wilson, 1990). Eusociality has enabled ants to invade and colonize much of the world.

Ant-aphid interactions

It was long believed that attendance of aphids by ants was entirely a mutualistic relationship, where both participants in the relationship receive a net benefit as opposed to other symbioses that only benefit one party (e.g. parasitism or commensalism). The basic relationship between ants and aphids is that ants provide protection against predators and/or parasitoids to aphids which in turn produce a rich food source (honeydew) for the ants (Addicott, 1979; Bishop & Bristow, 2001; Buckley, 1987a, b; Hopkins & Thacker, 1999; Rauch et al., 2002; Dutcher et al., 1999; Flanders, 1957; Flatt & Weisser, 2000; Renault et al., 2005; Bradley & Hinks, 1968). While in some systems this idea seems true, ant-aphid interactions are far more complex and there is a growing body of research that is showing everything from facultative to obligate relationships, and from mutually beneficial relationships to interactions laden with heavy fitness burdens incurred by both parties (Way, 1963; Bradley & Hinks, 1968; Breton & Addicott, 1992a, b; Cushman & Addicott, 1989; Cushman & Whitham, 1991; Fischer et al., 2001; Fischer, 2001; Kaneko, 2002, 2003; Kaplan & Eubanks, 2005; Offenberg, 2001; RicoGray & Castro, 1996; Sakata, 1994; 1995, 1999; Sakata & Hashimoto, 2000; Skinner & Whittaker, 1981; Stadler & Dixon, 1998; 1999; 2005; Stadler et al., 2001, 2002, 2003; Völkl, 1992; Völkl et al., 1999; Yao & Skimoto, 2001).

Degree of mutualism

Ant-aphid interactions range from very facultative encounters to completely dependent systems that appear coevolved. In a survey study, done in the Manitoba province of Canada, several interesting associations were made that included both obligate and facultative aphid tending (Bradley & Hinks, 1968). *Cinara pergandei*, amongst other *Cinara* species, was only sporadically tended by ants; however, *Cinara gracilis* had a much tighter association. When the ants (*Formica obscuripes*) were removed from a sympatric area, *C. gracilis* populations plummeted and the only colonies that survived had been “rescued” by neighboring interspecific ants (Bradley & Hinks, 1968). Another exclusion study, located in the Córdoba Province, Argentina, was conducted to ascertain not only the effects of aphid colonies when the ants were excluded but also the levels of aphid predators. When a species of *Campanotus* was removed from tending colonies of *Aphis coreopsidis* the aphids suffered heavy losses and as a corollary the number of aphidophagous spiders increased in the absence of the ants (Renault et al., 2005). A similar study was done looking at two mutualistic systems involving the ants *Lasius niger* and *L. fuliginosis* tending *Aphis serpylli* and *Stomaphis quercus*, respectively (Hopkins & Thacker, 1999). While exclusion experiments were not done in this study, Hopkins and Thacker (1999) looked at spatial distribution of the aphid colonies as a function of distance from their respective attendant ant nests. Their results showed a very high correlation with aphid colony occurrence increasing as distance from ant nest

decreased; similar distribution patterns and correlations have been documented in other systems as well (Dutcher et al., 1999; Tilles & Wood, 1982). Some ant species will transport hemipterans to optimal feeding or shelter locations. *Crematogaster lineolata* will actually move *Aphis lugentis* from the roots to the foliage of *Senecio douglasii* during the day and back again at night (Buckley, 1987a). Although not documented in the aphids, transportation has been taken to a new level in the mutualism of *Acropyga fuhrmanni* and *Eumyrmoccus* species of coccid. The ant's and coccid's biology are so intertwined that foundress queens will leave the parental nest and actually bring a coccid along to found not only a new colony for the ants, but a new colony for the coccid (Dixon, 1986; Flanders, 1957).

The degree of mutualistic interaction of the described systems seems predicated on the following: aphid mobility, alarm pheromone suppression and honeydew composition and amount (Way, 1963; Nault & Montgomery, 1976; Buckley 1987a, b). The more active aphids are and the more alarm pheromone produced seems to be negatively correlated with ant attendance. Honeydew production and its effects on ants is discussed below in terms of the associated costs. The literature is replete with examples demonstrating the true mutualistic nature of many ant-aphid systems, yet this interaction imposes several costs on the participants.

Costs of ant-aphid mutualisms

Principal disadvantage for the attendant ants is devoting members of the colony worker force to protect the aphids, exposing the ants to varying degrees of mortality, e.g. through predation or desiccation. The aphids provide an excretory product that would normally already be produced as a function of their feeding habits. In reality, the aphids undergo several changes in their feeding behavior and internal physiology to induce and maintain ant attendance. The major source of pressure for aphids is the composition, quality and amount of honeydew needed to sustain ant interactions.

Honeydew manipulation

The components of honeydew amount, release rate and individual sugar composition all play a role in shaping ant-aphid mutualisms (Völkl et al., 1999; Yao & Skimoto, 2001). Völkl and colleagues demonstrated the ant *Lasius niger* preferred trisaccharides over di- and monosaccharides, which correlated with differential tending of three different aphid species, *Metopeurum fuscoviride*, *Brachycaudus cardui* and *Aphis fabae*. *M. fuscoviride* produced a larger amount of honeydew with a significantly higher level of trisaccharides than the other two species. These aphids elicited more ants per unit time that would presumably offer better protection than the comparably sparse attendance of *A. fabae* and *B. cardui*. Specifically the trisaccharide, melizitose, has been thought to be principally an attractant for *L. niger* as it is not found in plant phloem and does not apparently serve any other known

function (Kiss, 1981). It has also been shown that aphids will increase their production of honeydew in the presence of ants in order to elicit tending (Stadler & Dixon, 2005).

Physiological changes

Increasing honeydew production and altering the sugar content of honeydew (if physiologically possible for the species) does not come without costs to many aphids (Völkl, 1992; Fischer, 2001; Stadler & Dixon, 1998; Stadler & Dixon, 2005; Stadler et al., 2001). Stadler (1998) identified various detrimental affects that aphids suffered as a result of altering honeydew production to suit their ant attendees: increased developmental time, delayed offspring production, smaller gonads, fewer well developed embryos and overall reduced growth rate. Most of these effects were consequences of having to produce higher quality honeydew for ant attendance and thereby reducing available nutrition for the aphid. Obviously these mutualisms would not persist if the fitness costs incurred outweighed the net fitness gain from the ant's protection (Stadler et al., 2001).

Although not many instances have been documented, the potential for interspecific competition for ant attendance is strong. In several systems where more than one aphid is on a single host plant there has been preferential selection by tending ants to defend the more preferred aphid and/or to prey upon the non-preferred species.

Examples of this preferential selection have been demonstrated with *L. niger* tending fava bean aphids as well as *Formica* sp. tending a combination of the following aphid species: *Cinara banksiana*, *Toumeyella parvicornis*, *Schizolachnus piniradiatae*, *Drepanosiphum platanoidis*, *Periphyllus testudinaceus*, *Operophtera* sp. (Bishop & Bristow, 2001; Cushman & Addicott, 1989; Fischer et al., 2001; Skinner & Whittaker, 1981). There is even evidence that individual members of a herd will compete for ant attendance, such as in membracids (Hemiptera: Membracidae) (Cushman & Addicott, 1989; Cushman & Whitham, 1991). The best example for intraspecific competition in aphids was documented in a system of experimentally manipulated populations of *Aphis varians* on fireweed, (*Epilobium augustifolium*). Cushman and Addicott restricted movement of *F. fusca* to heavily infested shoots of fireweed and monitored the populations and ant attendance of the restricted shoots as well as adjacent shoots with smaller *A. varians* populations. The ants were allowed to access the shoots after three days of exclusion. The results showed that ant presence increased significantly on the neighboring shoots, but once the restriction was removed, the previous level of ant tending resumed on the heavily infested shoots. While a simple occurrence, this preferential shift shows that ants are choosing amongst conspecifics in terms of sub population. Since aphid performance was correlated with ant attendance, larger populations of the same species were passively competing with smaller populations in the same location (Cushman & Addicott, 1989).

Ants can be great terrestrial protectors, but sometimes they do not provide adequate protection for their tended aphids from parasitoids or act as predators in some circumstances. A study looking at the defensive performance of *L. niger* and *Pristomyrmex pungens* tending *Aphis gossypii* demonstrated that the ants were very successful at deterring oviposition by a group of hyperparasitoids, but the primary parasitoid (*Lysiphlebus japonicus*), was able to oviposit with impunity (Kaneko, 2002; Kaneko, 2003). *L. niger*, one of the quintessential tending ants, has also been shown to systematically slaughter some of the aphid species they tend as is the case with herds of *Lachnus tropicalis* and *Myzocallis kuircola* (Sakata, 1995). While predation by ants is not something new, this study demonstrated that the ants were somehow assessing the number of aphids and kept an optimal number for honeydew production / protein for the ant colony (Sakata, 1994; Sakata, 1995). It has been hypothesized that ants can mark aphids they tend and/or extract honeydew from, which may deter intraspecific predation, i.e. the mark may chemically signal naive foraging conspecifics not to prey on the aphid (Hölldobler & Wilson, 1990; Sakata, 1994; Sakata, 1995). Other discrepancies in tending ability exist, but are harder to place into easily distinguishable patterns. Many studies have shown that ants reduce predation effectively only when aphids are either in smaller groups, larger groups or group size has no effect (Breton & Addicott, 1992a; Kaplan & Eubanks, 2005; Katayama & Suzuki, 2002; Sakata, 1995; Sakata & Hashimoto, 2000; Stadler et al.,

2003). While ant defense is a highly complex system, the consensus is that while the aphids provide attractive honeydew tending ants will provide some degree of protection (Way, 1963; Buckley 1987a, b).

Most of the current literature has focused on the direct effects of ant-aphid interactions, but there are several indirect effects that have emerged from these ecological studies. Kaneko (2003) uncovered an interesting indirect effect of reduced hyperparasitism. The primary parasitoid (*L. japonicus*) that was evading the ant defense, experienced inadvertent protection of the ants they evaded, because *L. niger* was doing a great job of fending off various hyperparasitoids of *L. japonicus* (the ants behaved as if this hyperparasitoid was going after the herd). In addition to hyperparasitism, reduced herbivory has been recorded as an indirect benefit to some plants hosting aphids tended by ants (Karhu, 1998; Vrieling et al., 1991; Floate & Whitham, 1994). This is an interesting trade-off considering the aphids are robbing the plant's nutrients from the phloem. Plant fitness benefits would only occur if the defoliation pressure was greater than the nutrient depleting activities of augmented aphid populations. In some cases augmented ant tended aphid populations reduced inflorescences and seed load of *Paullinia fuscescens* (Sapindaceae) (RicoGray & Castro, 1996). Even though the mechanisms are unclear, it does raise the possibility that plants in addition to hosting the ant's aphid herds might incur a variety of secondary positive and/or negative effects through ant-aphid mutualisms.

To reach even a modest level of understanding of the level complexity in ant-aphid interactions would take several life times of dedicated research. This brief overview demonstrates that ants and aphids do not engage in simple, straightforward mutualisms. Even classic textbook examples of both parties benefiting show costs being incurred upon closer observation. Much research is needed to fill in the gaps of the ecology, behavior and overall complexity of many if not all these tending relationships.

Invasive species:

Invasive species are quickly becoming one of the most important ecological problems facing countries worldwide, given the rapid increase in international commerce and travel. The aims of this review are to bring the reader up to speed with the relative importance of invasive species research, followed by a narrow focus on ant-invasions. There is vast literature on the various aspects of invasions by exotic species. For a more thorough account of the implications of invasions in a generalized and theoretical perspective I suggest the following reviews: Drake et al. (1989), Franklin et al. (1999), Liebhold et al. (1995), Sakai et al. (2001) and Tempel et al. (2004). Many of the key elements overlap in the conclusions of these reviews. These can be summarized by the following: 1) invasive plants establish in an area devoid of herbivore pressure which is aided by 2) the displacement of native herbivores by

invasive insects, both of which give you a massive combined biodiversity/species richness reduction factor.

Invasive plants

Many studies focused on the extreme competitive ability and importance of invasive plants (Attie et al., 2005; Cappuccino & Carpenter, 2005; Donlan et al., 2003; Knops et al., 1999; Parker et al., 2006; Thelen et al., 2005; Daehler, 2003; Van Auken, 2000). Daehler (2003) pointed out a particularly interesting native-invasive comparison of 79 pairs of plants. The results showed there was no difference between natives and exotics in terms of growth rate, competitive ability and fecundity. The key to invasive success lied in anthropogenic disturbances combined with an overall advantage in low cost foliar growth. Another common theme in the literature is how these exotic plants incurred significantly reduced herbivory when compared to their native counterparts (Attie et al., 2005; Cappuccino & Carpenter, 2005; Donlan et al., 2003; Parker et al., 2006). The Parker et al. (2006) study demonstrated a net loss in herbivory on exotics, but there were adequate numbers of native herbivores that could effectively consume the invasive plant material. These native herbivores, however would become displaced as a consequence of exotic species. This study illustrates the importance of looking at multiple trophic levels, when trying to make claims about invasive plant impacts.

Non-insect invasive animals

There are several other invasive taxa ranging from microscopic soil organisms to medium sized mammals (Amat et al., 2005; Belnap et al., 2005; Bohlen et al., 2004; Byers, 2000; Gundale et al., 2005; Johnson & Padilla, 1996; Smallwood, 1994; Van Auken, 2000). I will attempt to cite a few examples to give an idea of the breadth of invasive species importance. Amat et al. (2005), demonstrated that the American brine shrimp *Artemia franciscana* has invaded the Mediterranean area and completely displacing native shrimp species. There is an interesting example of exploitive competition by an exotic snail in northern California. The slow moving gastropod now dominates many of California's marshlands, causing many local extinction and causing major ecological disruptions (Byers, 2000). Earthworms have also found to be invasive species, completely altering the substrate in the environments and disrupting the lower trophic levels of communities where they occur (Bohlen et al., 2004; Gundale et al., 2005). One of the highly publicized invasive organisms is the zebra mussel (*Dreissena polymorpha*) is spreading its way into a large percentage of the United States freshwater ecosystems at an unprecedented rate (Johnson & Padilla, 1996).

Invasive insects

International commerce has spread several of the most invasive and ecologically destructive insects to introduced environments. While, it is impossible to ascertain

just how many exotic insects have entered the United States, especially given lag times, i.e. exotic species are not detected until they have reached a damaging threshold, which can take years (Crooks, 2005). A couple notable examples of recent and or current invasive insects that are having a major ecological impact are the gypsy moth (*Lymantria dispar*) and the hemlock wooly adelgid (*Adelges tsugae*), both of which are terrible forest pests. The former, an extreme generalist, has ravaged much of the Northeast US forests, whereas the latter is on the verge of driving the entire hemlock range into extinction (Hayes & Ragenovich, 2001; USDA, 2006). Other examples of invasive insects include those that have been purposefully introduced for biological control and have caused indirect problems on non-target species. One case is the introduced weevil *Rhinocyllus conicus*, brought in to control an invasive thistle, which has been very successful. However, the native endangered thistle, *Cirsium pitcheri* is shown to be just as suitable a host as the target exotic thistle for *R. conicus* (Louda et al., 2003; Louda et al., 2005; Rose et al., 2005). Even though there is the risk of non-target effects to any new introduced organism, some believe it is worth the ecological risk (Hoddle, 2004), and there have also been reports of non-targets actually benefiting from an introduced species (Brown, 2003).

Invasive ants

There have been almost no reports of beneficial side effects when comes to invasions by exotic ants. The notable exception is *Solenopsis invicta*, the red imported fire ant,

which has been shown to remove defoliators from cotton plants (Kaplan & Eubanks, 2005) and reduce tick populations by preying on engorged females (Tschinkel, 2006). *S. invicta*, however, is of great economic, ecological and human health importance as it is a highly prolific, aggressive, competitive, stinging ant (Tschinkel, 2006). Ants have never been intentionally released but that has not stopped some of the worst insect invasions ever described. Ants, being social insects, have a marked advantage over solitary species in their flexibility to overcome native resistance to invasions (Moller, 1996). Many researchers have reviewed the progress of ant invasions and almost all of them mention the invasive nature of the sugar ants of South America, known as the Argentine ants, *Linepithema humile* (Blancafort & Gomez, 2005; Christian, 2001; Collingwood et al., 1997; Holway et al., 2002a, b; Ingram et al., 2006; Jensen & Six, 2006; Sanders et al., 2003; Vega & Rust, 2001).

Argentine ants:

The Argentine ant has invaded six continents and countless islands (Holway et al. 2002). It's effects on local ant communities have been extensively documented. I will review aspects of the biology more less unique to the Argentine ant, followed by how these attributes have made it a successful invader, the abiotic factors that affect Argentine ant invasability, the extent of current invasions, as well as the effects on local fauna (primarily local ant populations). General ant biology has already been discussed but some biological and behavioral aspects of Argentine ants help explain

its competitive edge and will be covered here. Argentine ant colony structure is highly variable and temporary, it has been shown that as little as 10 individual ants and a queen are all that is required to establish a viable colony (Halley et al., 2005; Hee et al., 2000). It has been shown that these nests and modest propagule size are highly adaptive to anthropogenic disturbances, as seen through studies measuring Argentine ant occurrence as a function of distance to human disturbance (Carpintero et al., 2004; Human et al., 1998). It has been claimed by many that the key to Argentine ant dominance is sheer numerical superiority derived from a combination of high growth rate, lack of intraspecific aggression and low genetic diversity in introduced ranges (Buczkowski et al., 2005; Buczkowski & Silverman, 2005; Buczkowski et al., 2004; Holway, 1998b; Holway & Case, 2001; Holway & Suarez, 2004; Ingram, 2002; Keller & Fournier, 2002; Liang, 2000; Liang, 2001; Tsutsui & Case, 2001; Tsutsui & Suarez, 2003; Tsutsui et al., 2001; Giraud et al., 2002). In its native habitat, Argentine ants are found in large dense nests, with clearly identifiable boundaries with other colonies and with other species (Tsutsui & Case, 2001). However, in introduced ranges Argentine ants have formed tremendous supercolonies, spanning thousands of kilometers, in Europe and the United States (Buczkowski & Silverman, 2005; Buczkowski et al., 2004; Giraud et al., 2002; Tsutsui & Case, 2001; Tsutsui et al., 2001). Argentine ants also exhibit high levels of aggression in its native habitat, whereas in the introduced range there is very little aggression, especially in the >1000km expanses in the western US (this statement,

however, is somewhat a cyclical argument since aggression is the primary qualitative measure to differentiate colonies in the first place). Interestingly in the southeastern portion of the US, Argentine ant invasion is much more patchy and turns out that intraspecific aggression is much higher in this region (Vásquez and Silverman, 2008). Higher aggression combined by several corresponding genetic analyses provide strong support for the notion that invasion is fueled by, low intraspecific aggression and low genetic diversity (Buczkowski et al., 2005; Buczkowski & Silverman, 2005; Buczkowski et al., 2004; Giraud et al., 2002; Heller, 2004; Heller et al., 2006; Ingram, 2002; Tsutsui & Case, 2001; Tsutsui & Suarez, 2003; Tsutsui et al., 2001; Holway, 1999b). A study manipulating resource patches (Nonacs & Soriano, 1998) showed that Argentine ants will alter forager number depending on previous patch quality, e.g., if the first patch was of poor quality the colony will send out only a few foragers into the next unexplored patch. The question remains, however, as to how the Argentine ant populations are able to grow. Reduced genetic diversity, as mentioned, may explain reduced aggressive to conspecifics and provide some insight into its expansion ability, but why do introduced ranges allow for such invasion spread?

The answer could lie in various abiotic factors. Several studies have looked at moisture content and temperature of the air and soil, elevation, proximity to water and/or humans and plant coverage to better understand the susceptibility of

introduced ranges (DiGirolamo & Fox, 2006; Holway, 1998a; Human et al., 1998; Krushelnycky et al., 2005; Menke & Holway, 2006; Suarez et al., 1998; Thomas & Holway, 2005). Studies around the world have uncovered a variety of trends from the data. Argentine ants are highly sensitive to ground moisture. In both survey and manipulative studies, colonies were only able to invade novel areas and maintain dominance in the presence of high, continuous moisture levels (Holway, 1998b; Human et al., 1998; Krushelnycky et al., 2005; Menke & Holway, 2006). If moisture dropped below a certain point Argentine ants retreated from the area, even at very high colony density. Elevation was another trend, where ants confined themselves to lower altitudes, which is logical since elevation directly correlates with temperature, i.e., the higher the altitude, the lower the temperatures. Being of subtropical-warmer temperate origin it is reasonable to see the restrictions imposed by a cooler temperate climate.

While the ants may be somewhat restricted by temperature and moisture, they still seem to completely dominate the areas they infiltrate. Almost no native ants have been able to share an area once Argentine ants enter their environment (Cammell et al., 1996; Carpintero et al., 2004; Carpintero et al., 2005; Human & Gordon, 1999; Human & Gordon, 1996; Human et al., 1998; Kennedy, 1998; Miyake et al., 2002; Oliveras et al., 2005a; Oliveras et al., 2005b; Sugiyama, 2000; Visser et al., 1996; Thomas & Holway, 2005; Thomas et al., 2005). Much of the evidence for native ant

reductions was gathered using pitfall trap data and comparing species presence, but a few studies offered observations that the others did not detect. Human et al. (1996) showed that Argentine ants heavily preyed on native ant alates and Thomas et al. (2005) showed that Argentine ants foraging was restricted to the morning as native ants outcompeted them in the afternoon in Australia. Although restricted to morning foraging, Argentine ants usurped a disproportionate amount of resources vs. native ants in afternoon, yielding a net advantage for the Argentine ants (Thomas & Holway, 2005). The only actual native ants to more or less coexist with the Argentine ants have mainly succeeded because of their extremely small size (e.g., *Solenopsis molesta* Say, *Monomorium ergatogyna* Wheeler, *Paratrechina sakurae*) or arboreal nesting habits (*Camponotus vitiensis*) of native ants in parts of California and Japan (Touyama et al., 2003; Wetterer et al., 2001). Argentine ants in the south of Spain were able to completely dominate the arboreal ants, so nesting habit alone is not sufficient in all cases to avoid competitive displacement (Carpintero et al., 2005). Although many studies quantify the number of ants and the presence or absence of natives, few studies have been able to do a direct comparison of the competitive ability of Argentine ants versus native ants. There are studies that measured aggression to other ants at bait stations and few studies that have quantified tending ability (including defense from aphid predators) of Argentine ants (Holway, 1999a; Thomas & Holway, 2005; Altfeld & Stiling, 2006; Barzman & Daane, 2001; Martinez-Ferrer et al., 2003). The Argentine ant has often been implicated in

explosive outbreaks of honeydew producing Hemiptera (reviewed in Holway et al. 2002; Ness and Bronstein, 2004), and it is thought that a plentiful carbohydrate resource, such as honeydew, can fuel the high worker densities seen in Argentine ant infestations leading to local ant extinction (Davidson, 1997; Davidson et al., 2004; Ness and Bronstein, 2004; Holway et al 2002). There are few studies, however, demonstrating natural resource utilization between sympatric native ants and Argentine ant, which is perhaps not surprising given the propensity of invasive Argentine ant to displace most native ant species. By comparing honeydew resource utilization between Argentine ant and native ants we may gain insights into one possible mechanism (more effective mutualists) underlying the invasion success of the Argentine ant.

Proposal:

My research is broken down into five projects, which are presented as five separate chapters or manuscripts: 1) physiological advantages, 2) ant-aphid interactions, without ant-ant competition, 3) ant-ant competition, using a aphids as a competitive resource 4) aphid defense and 5) a field study to demonstrate a combination of the first four projects. Quantification of competition included the following measures, either by themselves or in combination: aphid population growth, aphid mortality, ant mortality, ant forager/tender number, crop size, mg sucrose/honeydew consumption and predator mortality. Discussed again in the procedures of each chapter, propagule

size for most of the experiments (Chapters 1, 2 & 3) will be 100 ants and one queen, as suggested by the results of the before mentioned study by Hee et al. (2000). For Chapter 4, ant number was varied to produce discrete ratios of native vs. invasive ants and in chapter 5, being a field trial, ant number was variable. Variability of ant number in the field is one of the biggest confounding factors in studies when trying to assess competition of a native vs. an exotic on the individual level. Chapters 1-4 allowed for a controlled number of ants to better understand the competitive ability of an invasive ant. Whether a difference was detected or not between the ants was important is to see if Argentine ant dominance was simply a numbers game that many researchers have claimed (Holway, 1999a; Holway & Case, 2001; Oliveras et al., 2005a). The organisms involved in this research were as followed: *Linepithema humile*, *Tapinoma sessile*, *Aphis gossypii*, *Myzus persicae*, *Toxoptera aurantii*, *Hippodamia convergens*, *Chrysoperla carnea* and *Aphidius colemani*, commonly known as the Argentine ant, Odorous house ant, Cotton aphid, Green peach aphid, Black citrus aphid, Convergent ladybird beetle, Green lacewing and “Aphid parasite” respectively.

Tapinoma:

There is not much literature on *Tapinoma sessile*, despite its economic importance in urban entomology (Buczkowski & Bennett, 2006, 2008). There are only a few papers that directly addressed behavior and/or food preferences of the odorous house ant,

which will be helpful in maintaining proper laboratory conditions for the lab colonies (Barbani, 2003; Duffield & Snelling, 2002; Fellers, 1987; Klotz & Reid, 1992; Milford, 1999; Smith, 1928; Wang & Brook, 1970). The best current information on the behavior and ecology of *T. sessile* came from work done by Buczkowski & Bennett, (2006, 2008). These studies gave insight and confirm what was already anecdotally observed: odorous house ants have polygynous, polydomous colonies that can grow quite large, they tend a variety of hemipteran resources, prefer high moisture, disturbed habitat and have close association with humans. All of these characteristics made the odorous house ant a prime native candidate to contrast with the Argentine ant. Unlike Argentine ants, odorous house ants are able to coexist and have highly overlapping foraging territories with other ants (Paulson & Akre, 1991). Although no evidence for *T. sessile*, a close relative, *T. simrothi* increased the output of honeydew from the black citrus aphid* as well as preferentially selecting individuals (*the aphid involved in the field study, see chapter 5) (Dartigues, 1992a, b; Dartigues, 1993).

Overview Projects/Chapters:

The purpose of chapter 1 was to see, in the absence of aphids, if Argentine ants have a physiological or biological advantage over odorous house ant in terms of sequestering/imbibing sucrose. Recruitment data, an estimation of worker crop size and sucrose consumption (short and long term) was measured. Chapter 1 served as a

baseline to the subsequent chapters. Chapter 2 sought the affect(s) the ants had on both myrmecophilic and non-myrmecophilic aphids alone and in combination in a predator free space, but without the two ants interacting. Aphid number and number of tending ants per unit time were measured. Chapter 3 assessed the relative defense abilities of both ants from flying and crawling predators and a parasitoid, both singularly in the lab and in combination in the field. Proportional change in aphid number and number of tending ants post predator/parasitoid introduction through time were measured. The purpose of the fourth chapter was two fold, one was to see how the ants compete over a common preferred resource (hemipteran honeydew), the other was to see the effect of invasive ant propagule (small parcel of workers and queens) size on a well-established *T. sessile* colony. The purpose of this last chapter (5) was to identify trends in the field. Luckily a scenario presented itself in the field on a holly hedge where Argentine ants were at one end and odorous house ants at the other, both of which were tending black citrus aphids. Aphid number, ant number/ presence and predator presence was recorded.

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Chapter 1: A comparative analysis of sucrose consumption and recruitment of Argentine ants and odorous house ants.

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Abstract:

Invasive ants are economically and ecologically important worldwide. Their success can be attributed to a variety of sources, including numerical superiority and resource domination. The Argentine ant, *Linepithima humile*, is amongst the world's top ant invasive species. *L. humile* has been observed in the field to dominate resources and have numerical superiority over native ants and other arthropods. However, quantification of *L. humile*'s advantage in the field has been problematic, as it is difficult to control for colony size, resource quality, and other important factors. Through a series of laboratory experiments using the odorous house ant, *Tapinoma sessile*, as a model native species we were able to compare the competitive advantages of *L. humile* (or lack there of). Our findings demonstrate that ecologically similar native ant workers sequester sucrose at the same rate both individually and as a group, but *L. humile* workers sustain a clear behavioral advantage through rapid recruitment.

Introduction:

Invasive ants are amongst the world's most successful exploiters of territories in which they are introduced (Holldobler & Wilson, 1990; Moller, 1996). It has been empirically demonstrated in the field that invasive ants out-compete local ants in wide variety of facets of their ecology (e.g. Holway, 1998a, Human & Gordon, 1996; 1999). The acquisition and domination of resources, particularly carbohydrates, is thought to be a principal advantage of invasive ants over their local competitors (Collingwood et al., 1997; Ness & Bronstein, 2004; Sanders et al., 2003). Among the studied invasive ants, there are three primary subfamilies within the Formicidae; the Myrmecinae, Formicinae and Dolichoderinae (Holway et al., 2002).

The Argentine ant, *Linepithema humile*, is a very ecologically important member of the Dolichodorinae, as it has invaded every temperate and subtropical climate worldwide. In the ant's origin of northern Argentina and southern Paraguay, they are known as "sugar ants" for their affinity of cane crops, Hemipteran honeydew and other sugar-based substances. *L. humile* are polydomous, polygynous ants that grow very large colonies in their native range and vast colonies (>1000km) in introduced ranges (Tsutsui & Case, 2001, Giraud et al., 2002). While there is a growing body of literature helping to explain how *L. humile* colonies have grown so large in their non-native habitats (Holway, 1998b; Holway, 1999a, b; Holway & Suarez, 2004; Holway et al., 1998; Tsutsui & Case, 2001; Tsutsui & Suarez, 2003; Tsutsui et al., 2001), there is very little empirical evidence to explain the lack of competitive ability

from native ants or other arthropods for *L. humile* colony expansion (Human & Gordon, 1999). Many researchers have speculated that the competitive advantage of *L. humile* is manifested by a combination of a superior worker population, resource domination, and physiology. *L. humile* possesses superior numbers over any sympatric native, but there is little evidence to explain why the natives have lost their ecological foothold. Given that *L. humile* has been introduced solely through human activity and not from a continuous influx from its source population, *L. humile* arrives in relatively small propagules. There must exist other reasons for the unabated advance of *L. humile*.

We attempt to quantify advantages apart from numerical superiority/inferiority of *L. humile* by controlling colony size and contrasting resource acquisition with an equally sized colony of native ants. We chose the native ant *Tapinoma sessile*, the odorous house ant, another member of the Dolichodorinae, which is particularly important given the inherent physiological advantages dolichoderines possess for liquid feeding (Davidson et al., 2004). *T. sessile* is a major urban pest especially in the northern United States (Buczkowski & Bennett, 2006, 2008; Hedges, 2000; Barbani, 2003). *T. sessile* shares many of the same biological and behavioral traits as *L. humile*, e.g., large polydomous, polygynous colonies, reproductive budding, and sugar affinity (Buczkowski & Bennett, 2006, 2008; Barbani, 2003; Smith, 1928). Given the similarities we feel *T. sessile* is a suitable counterpart to *L. humile* for valid comparative analyses.

We quantified a series of pairwise comparisons, including differences in sucrose consumption, foraging load and recruitment strategy. We measured sucrose consumption both initially and over an extended period of time. Our goals were to (1) quantify the relative consumption rate of the two ant species at a standardized food source and (2) differentiate the recruitment strategies at that resource. We expect *L. humile* to be able to sequester larger amounts of sucrose initially and over time as well as recruit to a resource faster than *T. sessile*. These experiments would enable us to tease apart the advantages of *L. humile* observed in the field and to ascertain the relative role competitive interactions play in the invasive dynamics of *L. humile*.

Methods:

Organisms:

L. humile and *T. sessile* were all maintained in an insect growth chamber (5.0m L x 2.8m W x 2.4m H) at 27°C and 70%RH. Ants were contained in a 75mm petri dish nest filled with grooved moist plaster. We fed ants 25% sucrose solution, artificial diet (Bhatkar & Whitcomb 1970) and freshly killed adult female cockroaches (*Blattella germanica*) ad libitum.

Initial feeding and Recruitment:

One hundred workers and 1 queen of each ant species (N=10) were held in Fluon-coated plastic containers (200mm x 250mm x 75mm) with single petri dish nests filled with

moistened, grooved plaster. Each nest was provided an opaque cover (75mm x 75mm). Ants were provided water (25 ml), but deprived of carbohydrates for 24 hours and then exposed to a 300 μ l droplet of 25% sucrose solution. The ants fed from the droplet for 30 minutes.

Sucrose consumption was recorded, by the change in droplet weight, after 30 minutes. We also recorded the number of ants feeding at the sugar droplet every minute for the duration of feeding. We also recorded the change in weight of 300 μ l sucrose droplets without ant access over 30 minutes to control for evaporation.

Long term feeding:

We established 10 replicates of 100 worker ants and one queen of each ant species as above. We provided each replicate with a 15 ml test tube of 25% sucrose solution plugged with a cotton swab horizontally positioned, which provides an adequate feeding substrate for the ants and retards evaporation. We measured sucrose consumption by weight after 10 days of feeding. We also measured the weight change of vials of sucrose solution without ant access to control for evaporation.

Load size:

We set up 15 worker ants of each species with nests of Petri dishes filled with plaster, water and freshly killed German cockroaches. The workers were deprived of sucrose for 24 hours. We weighed each starved worker using a Cahn 27 electronic balance prior to sucrose

exposure, allowed to feed on sucrose and then reweighed. The corresponding difference represented the individual ant's load.

Statistical analysis:

All data were analyzed using SAS 9.1.3 statistical package (SAS Institute, 2002).

Recruitment data were using analyzed using a repeated measures analysis under PROC MIXED with appropriate contrasts. Sucrose consumption was analyzed using an ANOVA model under PROC GLM.

Results:

There was no significant difference in sucrose consumption between the two ant species (Fig. 1, $F_{1,18}=0.242$, $p=0.72$ & Fig. 2, $F_{1,18}=0.242$, $p=0.66$) as well as no difference in the per capita sucrose load of individual ants of either species (Fig. 3, $F_{1,38}=0.242$, $p=0.62$). However, there was a striking difference in recruitment strategy between the two species (Fig. 4), with *L. humile* having a larger number of ants present at the droplet through time ($F_{1,396}=15.91$ $p<0.0001$).

Discussion:

Contrary to expectations, laboratory colonies of *L. humile* did not consume more resources than similarly sized *T. sessile* colonies either in the short term or long term feeding experiments. Given equal numbers of available foragers, *L. humile* does not appear to have

an advantage over *T. sessile* in sequestering carbohydrate resources. There also appears to be no morphological advantage of *L. humile* being able to hold more resources per forager, since we failed to detect any significant difference in worker load size. While the amount of acquired resources may not competitively differentiate this exotic ant from its native counterpart, the recruitment strategy of *L. humile* is strikingly different from *T. sessile*. Colonies of *L. humile* flood a new resource with large proportions of their foragers (upwards of 20% in some replicates) within a few minutes, while *T. sessile* dedicates only a modest percentage of foragers. Thus, our findings demonstrate that similar amounts of sucrose are being consumed by each species, but the recruitment strategies (at least initially) are very different between them.

This evidence provides valuable insights as to how *L. humile* could yield an advantage over a novel resource in a shared environment. By using their foraging strategy *L. humile* can overwhelm a food source with nearly a 4:1 advantage within the first five minutes (Fig. 4). This work confirms (partially) the prevalence of *L. humile* at baits in the field, (e.g. Holway, 1998).

While it is difficult to make generalizations given circumstances of laboratory studies, it is interesting that *L. humile* does not have an advantage in liquid sequestering over native ants when variables such as resource quality, distance to resource, and (most importantly) ant number are controlled experimentally. This suggests that total consumption may not be as important a factor as the strategy of consumption. Field studies have shown that *L. humile*

usurps bait stations through massive recruitment (e.g., Holway 1998a). Our findings suggest that it is not just large numbers, but proportion of workers devoted to the food source also. Future efforts should concentrate on better understanding the full extent of the *L. humile* foraging strategy to a standardized food source. Variables worth investigating would be distance to the food source, source quality, and using recruitment as the primary response.

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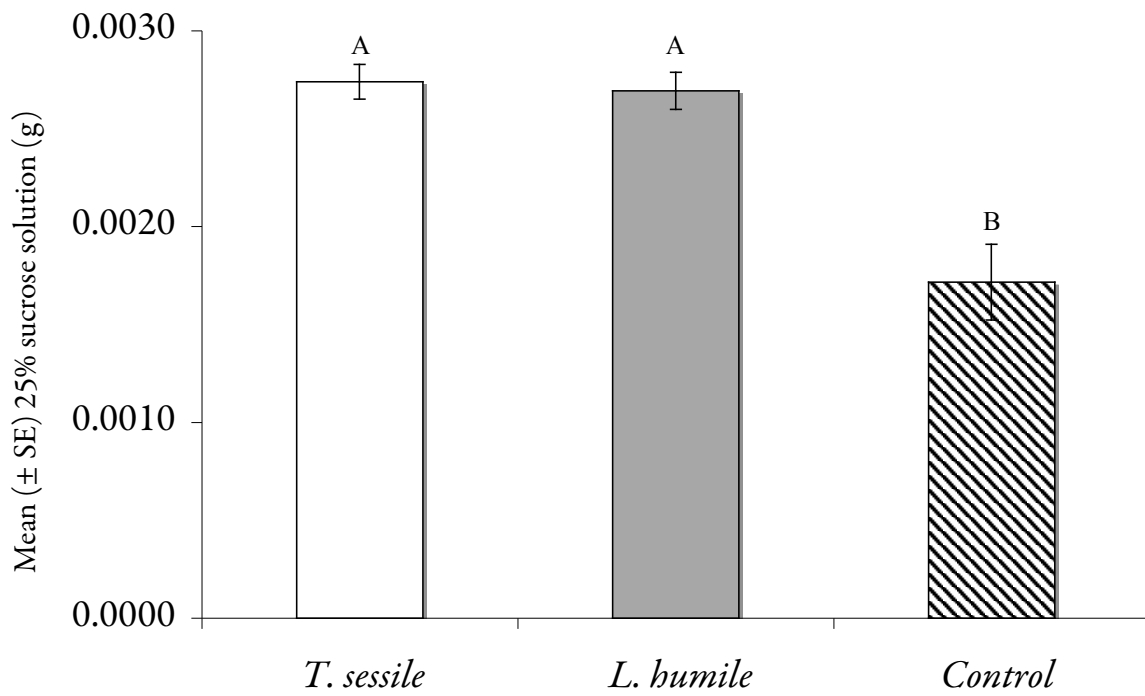


Figure 1: Mean (\pm SE) 25% sucrose solution consumed by either ant species or evaporated over a 30-minute period per minute, \square *T. sessile*, \blacksquare *L. humile*, \boxtimes Evaporative control. Different letters indicate significant difference at the 0.05 alpha level, SNK means groupings.

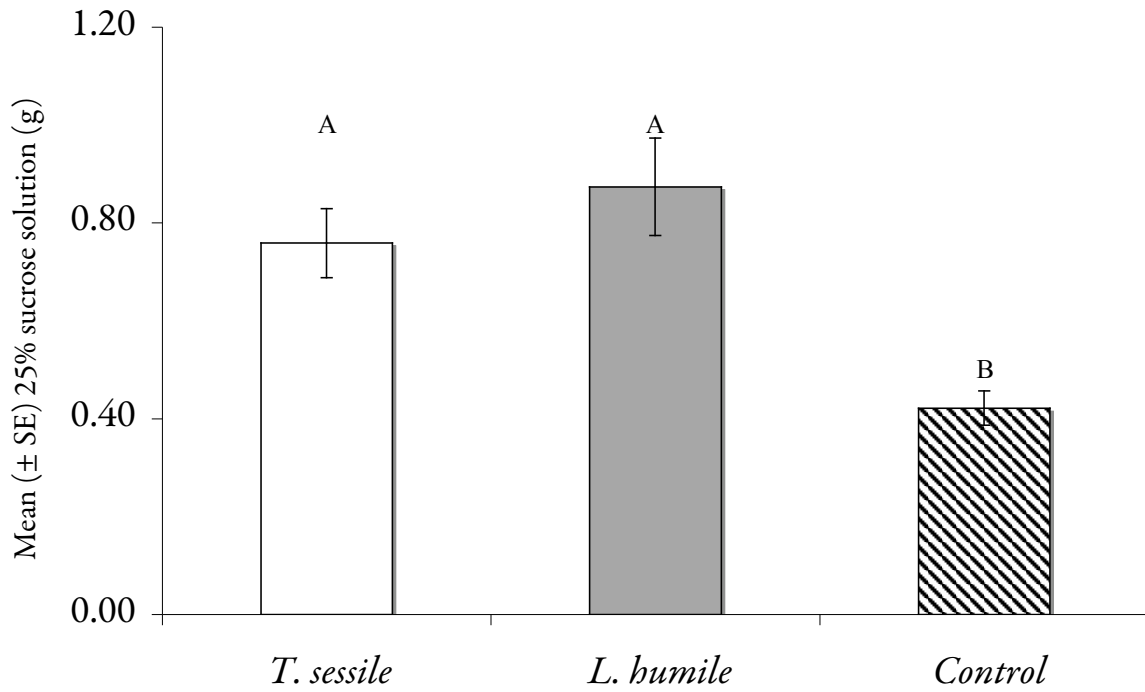


Figure 2: Mean (\pm SE) 25% sucrose solution consumed by either ant species or evaporated over a 10 day period per day, \square *T. sessile*, \blacksquare *L. humile*, \hatched Evaporative control. Different letters indicate significant difference at the 0.05 alpha level, SNK means groupings.

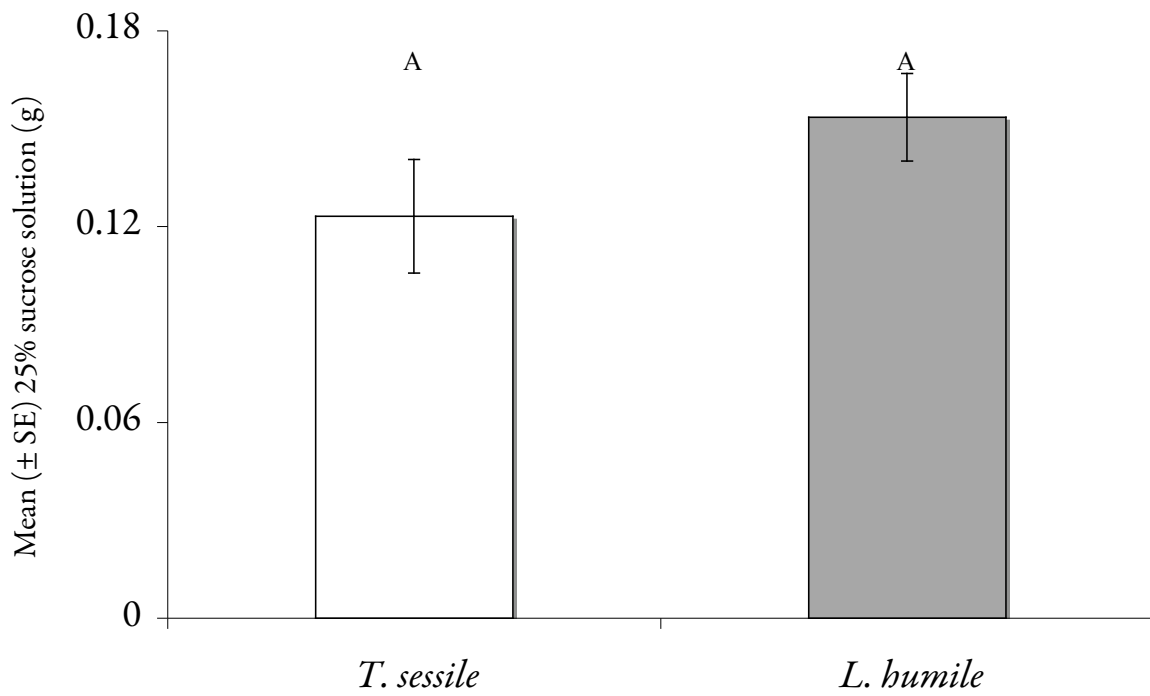


Figure 3: Mean (\pm SE) 25% sucrose solution consumed by either ant species after 24hr of starvation and allowed to feed to repletion, \square *T. sessile*, \blacksquare *L. humile*. Different letters indicate significant difference at the 0.05 alpha level, SNK means groupings.

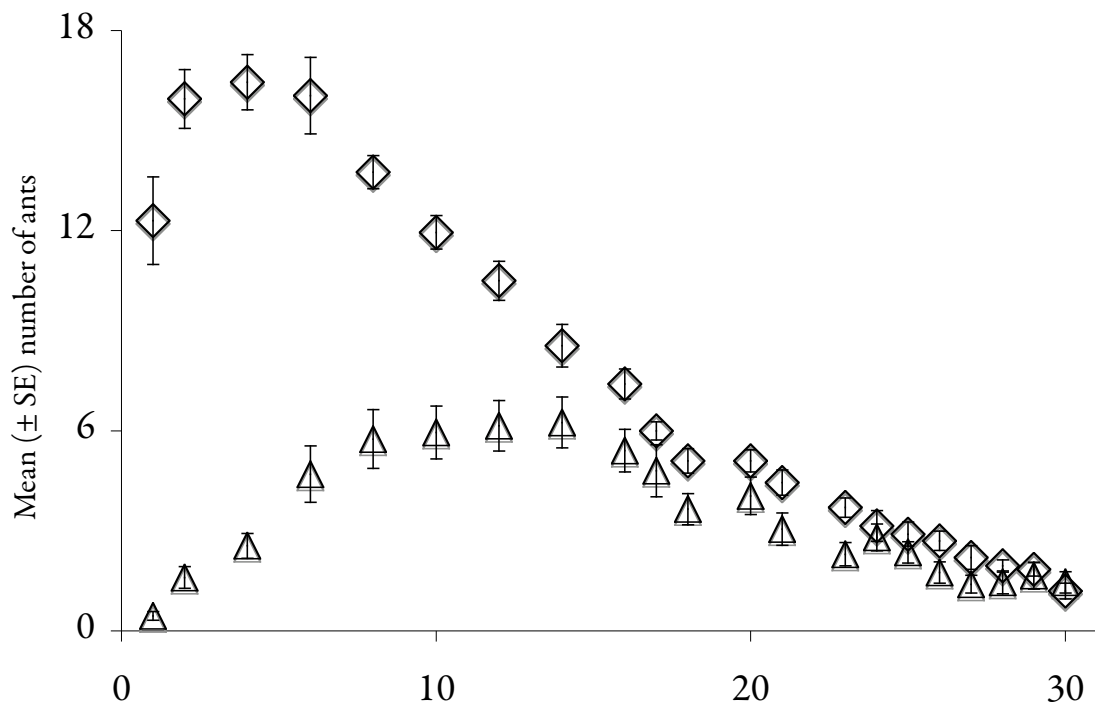


Figure 4: Mean (\pm SE) number of ants present at sucrose droplet over initial 30 min of feeding, \diamond *L. humile*, \triangle *T. sessile*.

Chapter 2: Population growth of *Aphis gossypii* and *Myzus persicae* in the presence of the ants *Linepithema humile* and *Tapinoma sessile*

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Abstract:

Interactions between honeydew-producing hemipterans and invasive ants can produce population increases in both symbionts with negative community level consequences. Yet, we do not know whether invasive v. native species are intrinsically better ant partners. We recorded hemipteran response to two Dolichoderine ants, *Linepithema humile*, a worldwide invasive species, and *Tapinoma sessile*, an ant native to North America. Populations of the myrmecophile, *Aphis gossypii* reached higher levels when exposed to *L. humile* than *T. sessile*. Also, *L. humile* foraging activity was greater than that of *T. sessile*, which may be responsible, in part, for the observed differences in *A. gossypii* numbers. Both ants species preyed upon a non-mymecophilic aphid, *Myzus persicae* when *A. gossypii* was also present, suggesting that these ants assess nutritional gains from honeydew vs. body tissue and eliminate less productive aphids competing for host plant space with a myrmecophile. We suggest that *L. humile* can facilitate honeydew production thereby fueling colony growth and leading to its numerical dominance in introduced habitats.

Introduction:

Invasive species are one of the most influential factors in reshaping natural ecological systems (Elton 1958). While rarely beneficial (e.g. Brown 2003, Tecco et al., 2006), invasive species generally have a dramatic impact on introduced ecosystems, not just causing biodiversity reductions via direct competition but also by using resources differently than native biota (Daehler, 2003; Dantonio and Vitousek, 1992; Franklin et al., 1999; Holway et al., 2002a; Liebhold et al., 1995). Invasive *Spartina spp.* reduce several species of native marine flora globally, but through increased salt tolerance, colonize larger portions of marine estuaries and reducing open mud flat habitat (Daehler and Strong, 1996). Arthropod composition on the mud flats can be greatly altered in the presence of some *Spartina spp.* and in some areas restrict foraging territory of shorebirds (Daehler and Strong, 1996). Similarly, zebra mussels, *Dreissena polymorpha*, are able to colonize harder substrates unused by native marine fauna, which results in severe reductions in phytoplankton and reduces available energy for higher trophic levels in the native community (Ludyanskiy et al., 1993). Apart from habitat resources, invasive species can also disrupt mutualisms including pollinator displacement, usurping valuable nectar and pollen resources; however, often not sufficiently replacing native pollinator roles (Traveset and Richardson, 2006). One of the most influential groups in altering mutualisms in communities are invasive ants.

Ants are arguably one of the most successful invading taxa on the planet, spreading through native ecosystems and disrupting mutualisms around the world (Ness and Bronstein, 2004;

Holway et al. 2002). *Linepithema humile* has extirpated most of the native ant fauna in fynbos shrublands in South Africa and assumed the role of seed dispersal for several plant species (Bond and Slingsby, 1984). However, *L. humile* did not adequately disperse seeds and caused the swift collapse of plant diversity in the area . On Christmas Island invasive *Anoplolepis gracilipes* has caused huge increases in hemipteran populations which have had cascading trophic effects, including reduced terrestrial arthropod abundance to reduced canopy growth (O'Dowd et al., 2003); subsequent removal of *A. gracilipes* resulted in the complete depletion of the explosive hemipteran populations (Abbott and Green; 2007). Given the boom and bust cycles that seem to follow ant invasion, invasive ant species may fundamentally engage in mutualisms (esp. hemipteran) differently than native ant counterparts.

There is some evidence that hemiptera will respond differently to the tending of native vs. invasive ants. Ness and Bronstein provide an overview of this difference and arguably the best example of hemipterans responding to exotic ants differently than native tending ants is Haines and Haines (1978) work on the Seychelles, where *A. longipes* tended *Ceroplastus rubens* and *Coccus viridis* were ca. 5-160 fold more abundant on citrus trees, than native *Technomyrmex albipes*. The mechanisms by which invasive ants differ from native ants are not known and most likely invasive ants behave differently than native species. Ants can alter hemipteran communities and populations, especially aphids by providing or withdrawing protection from natural enemies (e.g. and Whitham, 1991; Bradley and Hinks,

1968). The degree of ant attendance can be negatively correlated to aphid mobility, alarm pheromone production and comparatively lower honeydew quality and quantity than other aphid species in the area (Sakata and Hashimoto, 2000; Nault and Montgomery, 1976; Buckley, 1987a, b; Way 1963). Tending activity can also be affected aphid species, populations and individual clones (Sakata and Hashimoto, 2000; Sakata, 1994). For example, *Lasius niger* systematically marks some individuals of *Lachnus tropicalis* and *Myzocallis kuircola* for honeydew acquisition and others for consumption (Sakata, 1995). Thus, ants may remove unsuitable or non-mutualist aphid species or genotypes from their host plant, and thereby foster individuals/species that provide sustained benefits to the ants. Given the wide range of ant behaviors in response to potential aphid mutualists, ant species most likely exhibit different decision making processes. Perhaps native and invasive ants will choose to enter or respond differently to aphids, thereby causing shifts in aphid-community composition. Studies looking at the differences between native and invasive ants have two major obstacles for comparative analysis: 1) most studies are survey in nature comparing areas pre/post- or presence/absence of ant invasion, and 2) comparing tending effects of ants with unknown field population sizes.

We overcame these temporal and numerical obstacles by using a comparative model system in the laboratory. Our model consisted of discrete numbers of invasive and native ants exposed to a mutualist and non-mutualist honeydew producing aphid species both individually and in combination. In light of findings by O'Dowd et al. (2003) and Abbott

and Green (2007) we expected mutualist aphid populations to grow larger when tended by an invasive ant rather than a native. As mentioned above, ants may choose to shift tending behavior if more than one hemipteran is present (Sakata and Hashimoto, 2000) and potentially prey on hemipterans under certain conditions (Sakata, 1994, 1995) so a mixed aphid species treatment was performed. We expected non-mutualist aphids will either be ignored or preyed upon by both ant species, but the circumstances by which the decision to prey on the non-mutualist aphids may differ between the two ant species.

Materials and Methods:

Model organisms:

We chose *L. humile* as a model invasive species for the above mentioned characteristics as well as its successful global spread and aggressive nature (Rowles and O'Dowd, 2007; Holway et al., 2002). Probably the most ecologically and behaviorally similar native ant counterpart available with a sympatric range in North America is the odorous house ant, *Tapinoma sessile*. In addition to being in the same subfamily (Dolichoderinae) and relative size, these ants both form massive, polygynous, polydomous colonies, share the same nesting requirements and both have an affinity for hemipteran honeydew (Buczowski and Bennett, 2006; 2008). *Aphis gossypii* was chosen as a model mutualist aphid. *A. gossypii* is a widespread polyphagous honeydew-producing, ant-tended aphid (Blüthgen et al., 2006; Diaz et al., 2004; Kaplan and Eubanks, 2005; Vinson and Scarborough, 1989), which both *L. humile* (Mondor, 2008) and *T. sessile* (Powell, pers. obs.) will tend. *Myzus persicae* is

another widespread polyphagous aphid that excretes honeydew (Fisher et al., 1984; Hogervorst et al., 2007) but has never been reported to engage in mutualisms with ants and served as a non-mutualist aphid.

Organism maintenance:

L. humile, *T. sessile*, *A. gossypii* and *M. persicae* were all maintained in an insect growth chamber (5.0m L x 2.8m W x 2.4m H) at 27°C and 70%RH. Ants were contained in each with a 75mm petri dish nest filled with grooved moist plaster. We fed ants 25% sucrose solution, artificial diet (Bhatkar and Whitcomb 1970) and freshly killed adult female cockroaches (*Blattella germanica*) ad libitum. *A. gossypii* and *M. persicae* were obtained from BASF Co. (Research Triangle Park, NC) where they were reared on cotton, (*Gossypium barbadense*) and pepper, (*Capsicum annuum*), respectively. The cotton plants chosen for the experiments had 2-4 extra floral nectaries (EFN) throughout the course of the experiment [2 (max) on the cotyledons at the beginning of each experiment than a maximum of 4 at the end of the experiment]. While non-EFN (nectariless) cotton varieties are commercially available, we felt that the altered plant physiology may confound the plant's palatability to the aphid (e.g. Hardee and Bryan, 1997; Scrott et al., 1988; Sewart and Layton, 2000) and thus adversely affect honeydew production and quality. In light of work by Engel et al. (2001) where an EFN produced ca. two times the honeydew of a single aphid the starting populations in our experiments would begin with at least a 25-fold advantage of honeydew versus EFN production (see below). Even though EFN can have a significant effect on

certain ant-aphid interactions (Sakata and Hashimoto, 2000), the trend is far from universal (Del Caro and Oliveira, 1993). Aphids were transferred every 10 days to fresh plant material.

Verification of aphid species as mutualist and non-mutualist

Prior to conducting aphid population growth experiments we classified *A. gossypii* and *M. persicae* as either tended or untended by both *L. humile* and *T. sessile* using criteria similar to Volkl et al. (1999) and Fischer et al. (2001). We measured worker ant weight change, aphid mortality and the number of ants visiting plants with either aphid species. We measured worker weight change by collecting ants as they traveled from a nest at the plant base towards the leaves containing the candidate aphid as well as those traveling down the plant towards the nest. Individual ants were placed in preweighed gelatin capsules and weighed on a CAHN 27 electrobalance (Thermo Scientific, Beverly, MA). The corresponding difference in weight represented the average amount of honeydew sequestered by the ants traveling down the plant. We noted whether aphids were preyed upon and monitored changes in aphid populations. We recorded 20 worker weights for individuals both ascending and descending each of three separate host plants per aphid species for a total of 120 individuals per ant species.

Hemipteran response to ant attendance

One hundred workers and one queen of each ant species (N=10) were held in Fluon –coated plastic containers (200mm x 250mm x 75mm) with single petri dish nests filled with moistened, grooved plaster. Each nest was provided an opaque cover (75mm x 75mm). Ants were provided water (25 ml), artificial diet (100µl) and two freshly killed adult female *B. germanica* weekly. We transferred 100 aphids onto each host plant (ca. 150mm) rooted in plastic pots with soil (ca. 355ml). Both *L. humile* and *T. sessile* vacated the plaster nest and entered the base of each soil-filled pot within 24hrs. *A. gossypii* were transferred to cotton cotyledons revealing the first true leaf and *M. persicae* to pepper plants revealing the first two true leaves. We established two different controls: 1) both ant species foraging on aphid-free plants separately to account for non-specific ant activity on plants or ant activity not necessarily associated with aphid attendance, and 2) aphids on host plants with no ants present to measure the relative impact of ants on aphids under identical conditions. We counted all ants present on each experimental plant at one time each day. We recorded the number of aphids on each plant every 1-3 days for 3-4 weeks. Experiments were terminated when host plant health declined visibly (i.e. once plants lost leaves). We recorded ant mortality after drying out the soil in which the ants were nesting and counting ants that moved into an adjacent petri dish nests filled with moistened, grooved plaster. Drying the soil had little to no effect on ant mortality, (Powell, unpublished data).

We also conducted an experiment where ants had access to both *A. gossypii* and *M. persicae* at the same time to determine whether one aphid species would be handled differently by the ants when the other aphid species was present. Paired cotton plants with 100 *A. gossypii* and pepper plants with 100 *M. persicae* were held in containers with ants as described above (N = 10). We included controls with aphids but no ants. We recorded ant activity and aphid numbers as described above.

Statistical analyses:

All data were analyzed using SAS 9.1.3 (SAS Institute, 2002). Time series data (i.e. ant foragers and aphid population change) were analyzed using PROC MIXED with a repeated measures model, accounting for unequal time spacing, combined with relevant linear contrasts. Changes in ant weight and ant mortality were modeled using PROC GLM with an ANOVA model and SNK means groupings.

Results:

Aphid preference

Measurements of the weights of ants foraging up plants largely confirmed behavioral observations. Both *L. humile* and *T. sessile* workers were heavier when descending vs ascending plants with *A. gossypii* ($F_{1,28}=19.70$, $p<0.0001$, $F_{1,28}=20.51$, $p<0.0001$, Fig. 1), as would be expected were they tending aphids. In contrast, weights of *L. humile* and *T. sessile* descending plants infested with *M. persicae* were not different than those ascending these

plants, indicating that ants did not extract measurable amounts of honeydew ($F_{1,28}=0.01$, $p=0.92$, $F_{1,28}=0.19$, $p=0.66$, respectively, Fig. 1). After 48 hours over 90% of *M. persicae* had been removed from pepper plants by *T. sessile*, while *L. humile* did not significantly change *M. persicae* populations over time when *M. persicae* was the only aphid species present (Fig. 2). Aphid mortality was both reflected in the population data and by visual observation of aphid removal via *T. sessile* during the honeydew acquisition and hemipteran response experiments. Both ants sequestered honeydew from *A. gossypii* (Fig. 1) and no predation of *A. gossypii* was visually observed during any experiments or suggested in the aphid population data (Fig. 2, 3).

Do aphid populations respond differently to invasive and native ant partners?

Single aphid species exposure

L. humile and *T. sessile* both tended *A. gossypii*, however *L. humile*-tended *A. gossypii* numbers were ca. two-fold higher than *T. sessile*-tended *A. gossypii* ($F_{1,476}=51.50$, $p<0.0001$) (Fig. 2a). We did not observe *L. humile* interacting with *M. persicae* and numbers of *L. humile*-present *M. persicae* did not differ from numbers of untended *M. persicae* ($F_{1,264}=0.45$, $p=0.50$) (Fig. 2c). *T. sessile* were observed preying on *M. persicae* and consequently aphids were eliminated by day 4 ($F_{1,264}=80.17$, $p<0.0001$, Fig. 2c).

Mixed aphid species exposure

When ants had access to both *A. gossypii* and *M. persicae*, *A. gossypii* numbers again increased more with *L. humile* than *T. sessile* ($F_{1,294}=9.67$, $p=0.0021$, Fig. 3a) or without ants ($F_{1,294}=7.90$, $p=0.0053$, Fig. 3a). There was no difference in *A. gossypii* population change whether or not *T. sessile* was present ($F_{1,294}=9.67$, $p=0.0021$, Fig. 3a). In contrast to the neutral effect *L. humile* had on *M. persicae* in single aphid treatments, *M. persicae* numbers declined in *L. humile* treatments when *A. gossypii* were also present ($F_{1,294}=18.86$, $p<0.0001$, Fig. 3b). *M. persicae* population declines were greater in *T. sessile*-*A. gossypii* treatments than *L. humile*-*A. gossypii* ($F_{1,294}=9.51$, $p=0.0022$, Fig. 3b) or aphid-only treatments ($F_{1,294}=55.14$, $p<0.0001$, Fig. 3b).

Does the aphid-tending and foraging activity of invasive and native ants differ?

More *L. humile* and *T. sessile* were recorded on plants with *A. gossypii* than on *A. gossypii*-free plants ($F_{1,36}=18.21$, $p=0.0001$, $F_{1,36}=5.69$, $p=0.023$, respectively, Fig. 2b). The number of *L. humile* foraging on plants with *A. gossypii* was greater than the number of foraging *T. sessile* ($F_{1,36}=18.57$, $p=0.0001$, Fig. 2b). Pepper plants with and without *M. persicae* had few foraging *L. humile* and *T. sessile* ($F_{1,36}=3.37$, $p=0.075$, $F_{1,36}=1.15$, $p=0.29$, respectively, Fig. 2d). There was no difference in the number of *L. humile* and *T. sessile* on pepper plants ($F_{1,36}=1.14$, $p=0.29$, Fig. 2d). As expected, given the single (aphid) species trials, when ants had access to both *A. gossypii* and *M. persicae*, *L. humile* activity was

higher than *T. sessile* on cotton ($F_{1,36}=4.22$, $p=0.047$, Fig. 3c) but not on pepper ($F_{1,36}=0.05$, $p=0.82$, Fig. 3d).

Does ant survivorship differ among species or as a consequence of treatments?

At the end of the experiments there was no difference in the mortality of worker ants between ant-present treatments or controls among the *A. gossypii*, *M. persicae* and mixed aphid trials ($F_{3,36}=0.13$, $p=0.94$, $F_{3,36}=1.16$, $p=0.34$, $F_{3,36}=2.21$, $p=0.15$, respectively). Ant brood and queens were present in all replicates at the end of the experiments.

Discussion:

We provide the first evidence for differences in aphid population growth rates when tended by a native vs. invasive ant species. Differential growth of *A. gossypii* populations may be a response to ant foraging activity. The proportion of *L. humile* was threefold greater than the abundance of *T. sessile* on the tended aphid species. *L. humile* devoted roughly 6% of their worker force to tend *A. gossypii* vs. 2% for *T. sessile*. Increasing aphid populations may promote increased ant foraging and thereby create a synergy of positive reinforcement until aphid populations are at a spatial maximum.

Aphis gossypii increasing its reproductive output in response to the larger *L. humile* forager presence could be the result of both species optimizing fitness gain. Increasing aphid number may directly enhance fitness of the aphid colony (Turchin and Kareiva, 1989) and/or

indirectly by eliciting more ants for predator protection (Addicott, 1978b). However, increased density might lead to better predator awareness (e.g. Renault et al., 2005) and as hemipteran aggregations grow, ant defense ability can diminish (Harmon and Andow, 2007; Breton and Addicott, 1992). From the ant's perspective, devoting more worker resources to a fledgling hemipteran population may prolong the availability of the honeydew resource. Also, given the response of *A. gossypii*, other facultative mutualists may increase reproductive output through increased ant presence, providing additional benefits to ant species that commit larger numbers of tending workers. Hemipteran honeydew is a critical food resource for many ants (Davidson, 1997; Davidson et al., 2004; Tobin, 1994) and ants may manipulate hemipteran populations to optimize honeydew output (e.g. Banks and Nixon, 1958, Sakata, 1994, 1995). Therefore, sharp rises in *A. gossypii* numbers may be in response to specific interactions with *L. humile* and/or the frequency of these interactions. Since more *L. humile* than *T. sessile* were recorded on plants with *A. gossypii*, *L. humile* may not use a unique behavior(s) to stimulate aphid reproduction. Instead, aphids may respond to a threshold level of tending events. The few *T. sessile* on cotton plants may have been insufficient to stimulate increased *A. gossypii* reproduction. Experiments with greater numbers of *T. sessile* could address this issue.

Though *T. sessile* consumed *M. persicae* regardless of treatment, we recorded neither an increase nor decrease in *M. persicae* numbers in the presence of *L. humile* relative to the non-ant control in the single aphid species assay. While *M. persicae* might employ some

type of chemical camouflage (e.g. Akino, 2002; Akino and Yamaoka, 1998), it seems unlikely that such camouflage would be effective against only one of the ants in our system. Interestingly, when *L. humile* had access to both *A. gossypii* and *M. persicae* the latter was consumed, providing further evidence against chemical camouflage. Eventhough the aphids used different host plants, *L. humile* might have perceived *M. persicae* as competing with *A. gossypii* for host plant resources and by eliminating the untended aphid it improved the vigor of the tended aphid. This phenomenon could be better understood using two or more aphid species on the same host plant (e.g. Sakata and Hashimoto, 2000). *Lasius niger* consumes aphids that provide sub-optimal honeydew or unpalatable honeydew (Sakata, 1994; Sakata, 1995). Ants remove other insect herbivores from plants containing honeydew-producing hemipterans (Kaplan and Eubanks, 2005; Karhu, 1998) and take honeydew producers as prey items (e.g. *Drepanosiphum platanoides*, Skinner, 1980). Aphids can passively compete for ant tending services both inter- and intraspecifically (Addicott, 1978a, b) and under certain conditions ants will prey on both myrmecophilic and non-myrmecophilic aphids (Sakata, 1994; 1995; 1999; 2000).

Our work uncovers several ant-aphid interaction trends that past research has documented in the field, except we provide a native to invasive ant comparative model, with equivalent source populations. Prior experiments examining the impact of invasive ants, including *L. humile*, on hemipteran trophobionts use large established field populations (O'Dowd et al., 2003; Abbott and Green, 2007; Coppler et al., 2007; Ness and Bronstein, 2004) rendering a

direct comparison to native ants not possible. How do the invasive ant and hemipteran populations expand to the point where native ant-hemipteran mutualisms are eliminated? When introduced to a new habitat, invasive ant propagules are presumably small; smaller than established native ant colonies (Hee et al. 2000). Of the many characteristics shared by invasive ants we suggest that a disproportionately higher worker allocation over native ants to tending hemipterans stimulates the expansion honeydew-producing populations. These larger hemipteran populations can provide a greater carbohydrate resource base to fuel larger worker forces (Davidson 1997) offering greater competition and facilitating invasion.

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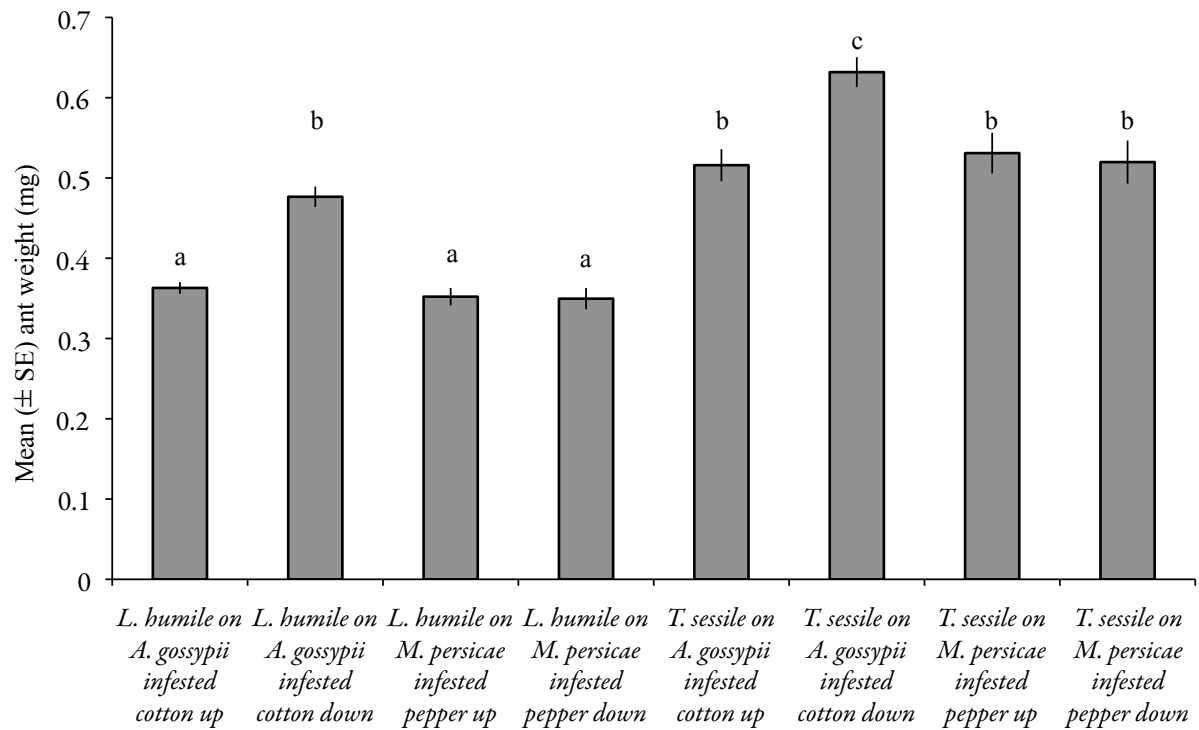


Figure 1: Mean (\pm SE) weight of ants traveling up and down pepper and cotton plants infested with *M. persicae* and *A. gossypii* respectively. Different letters represent significance below the 0.05 alpha level; (PROC GLM, ANOVA model, w/ SNK groupings).

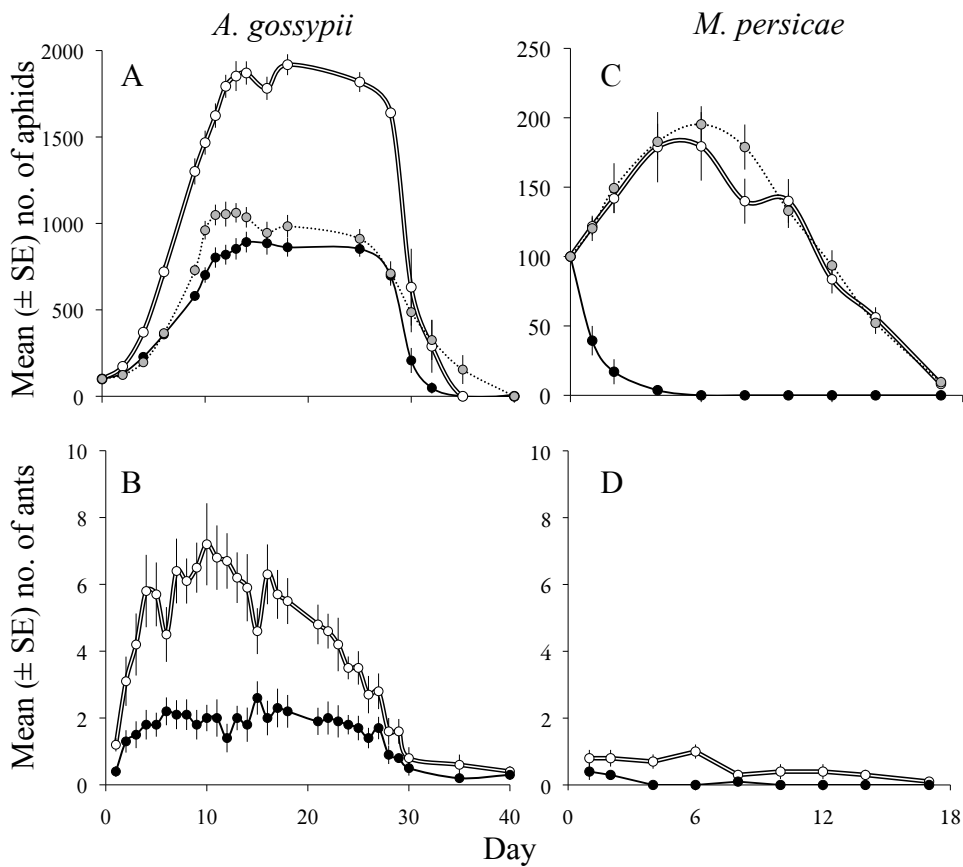


Figure 2: Effect of ants on aphid populations and ant activity in single aphid species assays. A) Mean (\pm SE) *A. gossypii* populations per plant over time. B) Mean (\pm SE) number of ants on cotton plants over time. C) Mean (\pm SE) *M. persicae* populations per plant over time. D) Mean (\pm SE) number of ants on pepper plants over time. \circ = *L. humile* and aphids present, \bullet = *T. sessile* and aphids present, \bullet = No ants present

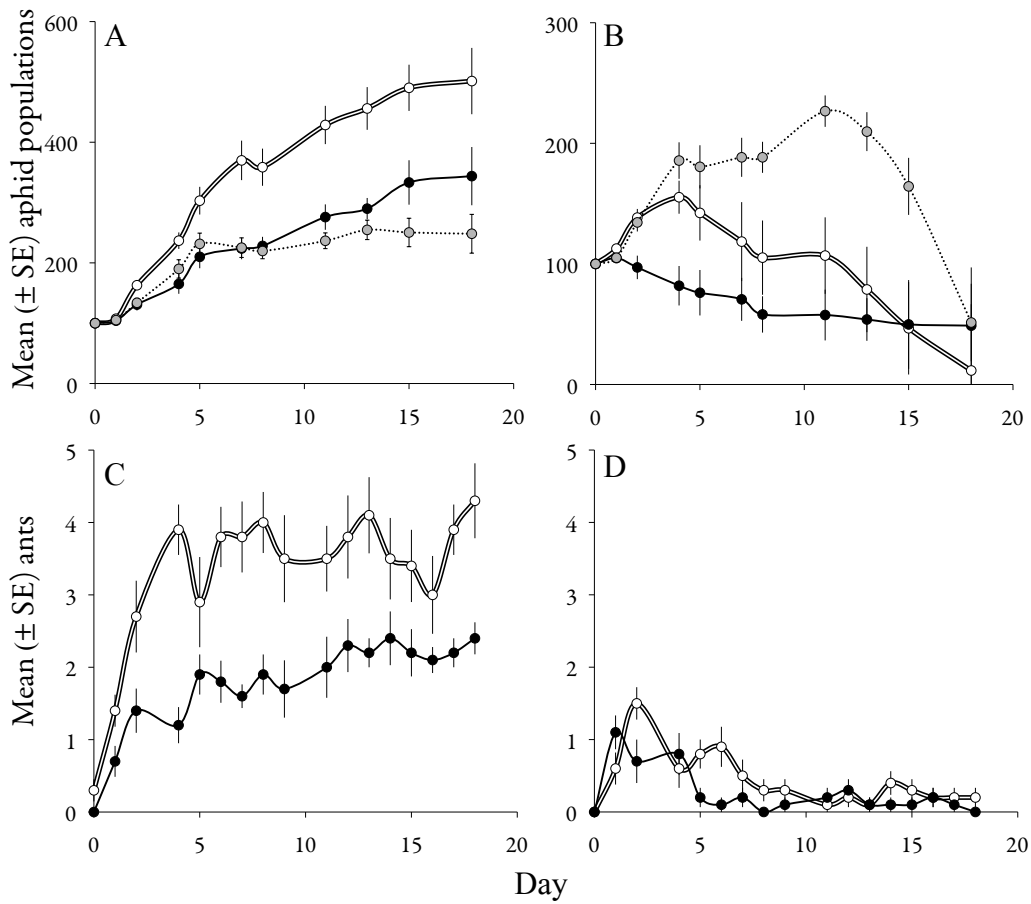


Figure 3: Effect of ants on aphid populations and ant activity in mixed aphid species assays. A) Mean (\pm SE) *A. gossypii* populations per plant over time. B) Mean (\pm SE) number of ants on cotton plants over time. C) Mean (\pm SE) *M. persicae* populations per plant over time. D) Mean (\pm SE) number of ants on pepper plants over time. \circ = *L. humile* and aphids present, \bullet = *T. sessile* and aphids present, \bullet (grey) = No ants present

Chapter 3: The effect of a native and invasive ant on *Aphis gossypii* populations subjected to natural enemy attack

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Abstract

Ants frequently protect honeydew-excreting hemipterans from their natural enemies in exchange for food. Invasive ant species have been reported to enter into mutualisms with hemipterans with a positive feedback mechanism presumably fueling the population growth of both partners and contributing to the displacement of native ants. Invasive ants are generally regarded as more aggressive than native ants and consequently they may be more effective at protecting honeydew-producing hemipterans from natural enemy attack. We tested this hypothesis in laboratory and field experiments with the invasive ant, *Linepithema humile*, and *Tapinoma sessile*, native to North America. We compared changes in populations of *Aphis gossypii* tended by equal numbers of these ants and exposed to the natural enemies *Chrysoperla carnea*, *Hippodamia convergens* and *Aphidius colemani*. While more *L. humile* than *T. sessile* workers foraged on plants with *A. gossypii*, both ants were equally effective at protecting *A. gossypii* from their natural enemies when aphid populations were relatively low. Using larger starting populations, *A. gossypii* increased faster when tended by *L. humile* v *T. sessile* and exposed to either *C. carnea* or *H. convergens*. *A. gossypii* numbers declined at the same rate when tended by *L. humile* or *T. sessile* in a field experiment. We conclude that due to its higher foraging tempo *L. humile* may more effectively patrol larger plant surfaces supporting honeydew-producing hemipterans than *T. sessile*, which may be a mechanism underlying the success of this and perhaps other invasive ants.

Introduction:

Ant-hemipteran mutualisms represent keystone interactions having broad, community-level consequences (Styrsky and Eubanks 2007; Way, 1963). These mutualisms may be obligate or facultative with hemipteran trophobionts providing ants fuel, in the form of honeydew, and receiving ant services in return, most notably protection from natural enemies (Altfeld and Stiling 2006; Breton and Addicott, 1992; Beattie 1985; Offenberg, 2000; Nixon 1951). Exclusion treatments in the field have verified to some extent the relative contribution that ants provide to hemipteran defense (Addicott, 1978a, b; Buckley, 1987a, b; Bishop and Bristow, 2001; Renault et al., 2005). Among native ants, it is generally the highly aggressive and territorial species that provide the best protection of honeydew-producing hemipterans (e.g. Buckley and Gullan 1991).

Invasive ant species share several traits, including aggressiveness, which are thought to contribute to their dominance and the displacement of native ants (Holway et al. 2002). The elimination of native ants by invasive ants may alter the specificity and effectiveness of mutualisms and as a consequence disrupt ecosystem function. Furthermore, mutualisms may also contribute to the establishment and expansion of ant invasions by fueling population growth (Simberloff 1999, Ness and Bronstein 2004). We do not know, however, whether the aggressive behavior intrinsic to invasive ant species makes them more effective than native ants at protecting hemipteran mutualists from their natural enemies.

Large outbreaks of sometimes seemingly inconspicuous hemipterans, are often correlated with the presence of invasive ant species, most notably *Anoplolepis gracilipes* (=longipes) (Abbott and Green 2007; O'Dowd et al., 2003 Haines and Haines, 1978), *Wasmannia auropunctata* (de Souza et al., 1998) and *Linepithema humile* (Altfeld and Stilling 2006, Barzman and Daane 2001). Honeydew-producing hemipteran outbreaks following native ant displacement by invasive ants may be a function of better protection against natural enemy attack from enhanced patrolling of the hemipteran habitat by a larger worker force, or more frequent and/or aggressive encounters with natural enemies by comparable size native and invasive ant colonies. Herein, we evaluate the impact of a native and invasive ant species on a hemipteran trophobiont and some of its natural enemies in laboratory and field experiments with ant colonies of identical size and composition.

The Argentine ant, *L. humile* (Formicidae: Dolichoderinae) is among the most destructive and well-studied invasive ant species (Holway et al., 2002; Silverman and Brightwell, 2008). Native to portions of South America, it has spread throughout the world to regions with Mediterranean, subtropical and mild-temperate climates (Suarez et al., 2001). Once established *L. humile* readily displaces most native ant species and other arthropods with community-level consequences (Holway et al., 2002; Holway 1998; Touyama, et al., 2003; Human and Gordon, 1996, 1999; Oliveras et al., 2005). A large part of the Argentine ant's invasion success lies in the formation of extensive supercolonies with interconnected nests covering large areas (Holway et al. 1998; Giraud et al., 2002; Tsutsui et al., 2000). Through

suppression of aphidophagous organisms, Argentine ants are responsible for large increases of honeydew-excreting plant pests, e.g. *Aphis coreopsidis* (Altfeld and Stilling, 2006) and *Saissetia oleae* (Barzman and Daane, 2001). Native ants have also been shown to sustain large damaging hemipteran populations in the field on *Salix sp.* (Sipura, 2002) and some agricultural systems, e.g. *Lasius niger* and the rosy apple aphid, *Dysaphis plantaginea* in a U.K. orchard (Stewart-Jones et al. 2008).

During its establishment within the introduced range *L. humile* has dominated and displaced most native ant species, including those that rely heavily on hemipteran honeydew (Ward and Harris, 2005; Holway, 1998, Human and Gordon, 1996). One of these displaced ants *Tapinoma sessile* (Formicidae: Dolichoderinae) is native to North America and shares many traits with *L. humile* (e.g. worker size, polydomy, polygyny, vagility and omnivory, including hemipteran tending) (Barbani 2003; Smith, 1928, Buczkowski and Bennett 2006, 2007; Passera, 1994). Given the biological and ecological similarities between *L. humile* and *T. sessile*, we contend that a direct comparison of their interaction with honeydew producing hemipterans and their natural enemies is appropriate.

Of the numerous honeydew-producing hemipterans that could be utilized by both *L. humile* and *T. sessile* we chose to study the interactions of *Aphis gossypii* with both ants. *A. gossypii* is highly polyphagous having been found on over 90 plant families in 40 orders, including important agricultural products such as cotton, avocado, cucumber, papaya, eggplant (Ebert

and Cartwright 1997). Furthermore, *A. gossypii* is easily reared in the laboratory (Vinson and Scarborough, 1989) and engages in mutualisms with ants (Diaz et al., 2004; Kaplan and Eubanks, 2002; Vinson and Scarborough, 1989), including *L. humile* (Mondor et al., 2008) and *T. sessile* (BP personal observation).

A. gossypii is attacked by numerous predators and parasitoids (Ebert and Cartwright 1997; Zamani et al., 2006; Zamani et al., 2007; Zarpas et al., 2007). Kaplan and Eubanks (2002) demonstrated that *Solenopsis invicta* (another invasive ant from South America) effectively suppressed both *Chrysoperla carnea* (Neuroptera: Chrysopidae) and *Hippodamia convergens* (Coleoptera: Coccinellidae) larvae predation of *A. gossypii* in greenhouse experiments. We selected natural enemies of *A. gossypii* exhibiting different behaviors &/or modes of dispersal for our experiments, in which to identify possible ant-specific responses. The natural enemies were a non-flying predator, larval *C. carnea*, a flying predator adult *H. convergens* and a parasitoid *Aphidius colemani* (Hymenoptera: Aphidiidae). Since *L. humile* is generally more aggressive than *T. sessile* (Buczowski and Bennett, 2007), we predict that *L. humile* would better defend *A. gossypii* from natural enemy attack, which may be a key component of its invasion success.

Methods:

Organisms:

Source populations for *T. sessile* and *L. humile* were taken from several collections of multiple, discrete geographic, urban locations. *L. humile*, *T. sessile* and *A. gossypii* were all maintained in culture in the laboratory at 27°C and 70%RH. *T. sessile* and *L. humile* were confined in fluon-coated trays (200mm x 250mm x 75mm) and provided petri dishes nests (150mm diam) filled with grooved plaster. Ants were fed 25% sucrose, artificial diet (Bhatkar and Whitcomb, 1970) and freshly killed German cockroaches, *Blattella germanica* (Blatteria: Blattellidae). *A. gossypii* were obtained from (Company, city, state) and reared on ca. 50 cotton (*Gossypium sp.*) cotyledons (ca. 2 weeks old) in plastic containers (150mm diam) w/ moisture-control potting soil. Aphids were transferred every 10 days to fresh plants. *C. carnea* and *H. convergens* were obtained from Biconet (Brentwood, TN) and *A. colemani* was obtained from Arbico Organics (Tucson, AZ). *C. carnea* were received as eggs and were subsequently raised on *A. gossypii* until the beginning of the third stadium (length ca. 4mm). Early stadium lacewing larvae are not effective predators of aphids when ants are present (Grover et al. 2008). *H. convergens* were received as adults and females were immediately used in our experiments. *A. colemani* arrived as mummies (parasitoid pupae encased in dead aphids), which were separated into gel capsules (#2, ca. 10mm in length) until adult emergence. After emergence the wasps were sexed and randomly paired. Copulation was observed in all pairings and female wasps were used in experiments 24 hours after the initial pairing.

Laboratory assessment of aphid protection by ants

Ants (100 workers and 1 queen) were placed in Fluon®-coated plastic containers (200mm x 250mm x 75mm) with Petri dish nests (75mm) filled with grooved plaster covered with a piece of linoleum. Ants received water (25mls), artificial diet (ca. 100µl) and two freshly killed adult female *B. germanica*, weekly. We transferred a mixture of 100 nymphal and adult *A. gossypii* to individual cotton cotyledons (ca. 75mm ht.) held in 473ml plastic cups. Ten replicates were performed per ant species per treatment. Each natural enemy species assay was performed consecutively. Treatments were as follows 1) aphids plus ants plus natural enemies, 2) ants plus aphids without natural enemies, 3) aphids plus natural enemies without ants, 4) aphids without ants or natural enemies. The number of ants and aphids on the plants was recorded once daily. After initial aphid transference, a single predator or parasitoid was released onto the plant. Treatments receiving *H. convergens* or *A. colemani* were enclosed in a wooden framed cage (300mm x 300mm x 600mm) surrounded by 150µm² viral mesh. We recorded aphid numbers and ant foraging activity until *C. carnea* pupation, adult *A. colemani* progeny emergence or if aphid numbers reached zero in control treatments with no ants, but predators present. We also recorded ant worker survival at the end of the experiment.

Aphid population size can influence an ant colony's ability to protect its mutualist partner against natural enemy attack (Breton and Addicott, 1992). Therefore, we conducted the above experiments again, however, allowing for aphid population growth prior to natural

enemy release. Once the aphid population growth rate started to slow and approach zero (ca. 10-20 days, depending on the trial) we released natural enemies, except for *A. colemani*, given the limited amount of aphids a single wasp can parasitize (Zamani et al. 2006, 2007).

Field assessment of aphid protection by ants

We examined the ability of *T. sessile* and *L. humile* to protect *A. gossypii* from a suite of naturally-occurring natural enemies in a field planted with cotton. We conducted our trial within a 10m² plot in Clayton, NC (NCSU research station) using plants (ca. 30cm ht) in five rows spaced 60 – 70 cm apart. Each plant (15 replicates) received 100 workers and one queen of either ant species housed in a 50ml screw-capped plastic centrifuge tube one-half filled with water and plugged with cotton. The lid was fitted with plastic tubing (200mm x 2 mm id x 4mm od). The containers were submerged at a 45° angle ca. 200mm within the soil adjacent to each plant (Fig. 1). Tubing from the containers was connected to the main stem of the cotton plant ca. 75mm above the soil surface. We also affixed a 1.5ml microcentrifuge tube filled with 100µl of artificial diet to the same junction point to provide additional nutrition for the ants. A semi-permeable shade cloth was placed on the ground within the plot, which allowed water from rainfall to penetrate, yet prevented soil from splashing onto the plants. A band of Tanglefoot® (ca. 50mm) below the end of the affixed tubing prevented ant escape. We placed 1000 aphids onto the newest plant growth. *A. gossypii* survival without ants, predators or parasitoids was determined on plants covered with 150µm² viral mesh. Aphids were counted daily for eight days, at which time aphids were no longer

detected on any plants. We counted the number of the ants and recorded any potential aphid predators present on plants. Data were recorded the same time each day (0800-1000 hours) (high midday-afternoon temperatures retarded all ant/predator activity). Treatments in this experiment were 1) *L. humile* or *T. sessile* and natural enemy access to *A. gossypii* 2) Natural enemy access to *A. gossypii* w/ supplemental diet and Tanglefoot, to ascertain any effect the supplemental diet had in attracting natural enemies and to see if the tanglefoot kept predators away 3) Natural enemy access to *A. gossypii* 4) *A. gossypii* only (natural enemy or ant access excluded by viral mesh).

Statistical Analysis:

All data sets with the number of ants and proportional change in aphids through time were analyzed in SAS 9.1.3 using a PROC MIXED repeated measures model with individual treatment contrasts (SAS Institute, 2002). Ant numbers were square-root transformed and proportional change in aphid number was log transformed for normality. The ratio of parasitized *A. gossypii* exposed to *A. colemani* was arcsine square-root transformed and *A. gossypii* populations exposed to *C. carnea* larvae for two hours were analyzed in SAS 9.1.3 using a PROC GLM model with Student-Newman-Keuls grouping for significance at the 0.05 alpha level (SAS Institute, 2002).

Results:

When pooled across all lab experiments worker mortality of *L. humile* ($18.9 \pm 1.06\%$ SE) and *T. sessile* ($19.4 \pm 1.23\%$ SE) was not significantly different ($F_{1,198}=0.09$, $p=0.76$). Also, 98% of *L. humile* and 97% of *T. sessile* queens were recovered in all laboratory replicates. In the field experiment, *L. humile* worker mortality ($72.7 \pm 7.51\%$ SE) did not significantly differ from *T. sessile* worker mortality ($65.4 \pm 8.53\%$ SE) ($F_{1,28}=0.42$, $p=0.52$) and 93% *L. humile* and 87% *T. sessile* queens were recovered at the end of the experiment. Though not counted, brood of both ant species was present at the completion of all experiments.

Aphid predation by *Chrysoperla carnea*

Plants with 100 A. gossypii

Over twice as many *L. humile* than *T. sessile* were counted on the plants irrespective of whether *C. carnea* were present ($F_{1,36}=2.86$, $p=0.0023$, Fig 2A), yet both ants affected *C. carnea* predation equally ($F_{1,18}=2.86$, $p=0.10$, Fig 2A). There was no significant change in recruitment in response to *C. carnea* by either ant species (*L. humile*: $F_{1,36}=0.04$, $p=0.84$, *T. sessile*: $F_{1,36}=0.10$, $p=0.75$ Fig 2A). When *C. carnea* were added to plants nearly all aphids were consumed within 2 hours (Fig 2A). *A. gossypii* survival was higher when *L. humile* ($F_{1,18}=66.37$, $p<0.0001$) or *T. sessile* ($F_{1,18}=96.79$, $p<0.0001$) foraged on plants with *C. carnea* than when ants were absent (Fig 2). Ant protection, however was not absolute, as *A. gossypii* numbers declined when both ants and *C. carnea* were present (*L. humile*: $F_{1,18}=94.43$, $p<0.0001$, *T. sessile*: $F_{1,18}=18.62$, $p<0.0001$).

Plants with higher initial numbers of A. gossypii

Again, more *L. humile* than *T. sessile* workers were on the plants prior to *C. carnea* introduction ($F_{1,36}=14.84$, $p=0.0005$, Fig 2B), and ant numbers did not change after *C. carnea* was introduced (*L. humile*: $F_{1,36}=0.61$, $p=0.44$, *T. sessile*: $F_{1,36}<0.01$, $p=0.99$, Fig 2B). Compared with treatments without *C. carnea* (Fig 2B), *C. carnea* reduced *A. gossypii* numbers without ants ($F_{1,225}=65.12$, $p<0.0001$) with *L. humile* ($F_{1,225}=43.17$, $p<0.0001$) or with *T. sessile* ($F_{1,225}=66.19$, $p<0.0001$, Fig 2B). Despite the losses, *L. humile* offered a significant amount of protection for *A. gossypii* ($F_{1,225}=5.57$, $p=0.019$), but *T. sessile* did not ($F_{1,225}=0.03$, $p=0.86$, Fig 2B). A direct comparison of defended *A. gossypii* also shows a difference between the two ants ($F_{1,225}=4.80$, $p=0.029$, Fig 2B). *C. carnea* larvae remained in the treatments until pupation, approximately five days after initial release.

Aphid predation by Hippodamia convergens

Plants with 100 A. gossypii

More *L. humile* than *T. sessile* were counted on the plants irrespective of whether *H. convergens* were present ($F_{1,36}=6.81$, $p=0.013$, Fig 3A), yet both ants affected *H. convergens* predation equally ($F_{1,228}<0.01$, $p=0.98$). Again, there was no change in recruitment in response to *H. convergens* by either ant species (*L. humile*: $F_{1,36}=0.93$, $p=0.34$, *T. sessile*: $F_{1,36}=1.70$, $p=0.20$). After *H. convergens* were placed on plants nearly all aphids were consumed within 3 days (Fig 3A). *A. gossypii* survival was higher when *L. humile*

($F_{1,228}=46.87$, $p<0.0001$) or *T. sessile* ($F_{1,228}=47.23$, $p<0.0001$) foraged on plants with *H. convergens* (Fig 3A). There was no effect of *H. convergens* on tended *A. gossypii* populations of either ant (*L. humile*: $F_{1,228}=0.18$, $p=0.67$, *T. sessile*: $F_{1,228}=0.01$, $p=0.94$, Fig 3A).

Plants with higher initial numbers of A. gossypii

Compared to treatments without *H. convergens*, *H. convergens* reduced *A. gossypii* numbers without ants present ($F_{1,408}=140.70$, $p<0.0001$, Fig 3B) or with *T. sessile* ($F_{1,408}=8.73$, $p=0.0033$, Fig 3B) but not with *L. humile* ($F_{1,408}=0.90$, $p=0.34$, Fig 3B). The impact of both ants on *H. convergens* predation was similar ($F_{1,408}=2.07$, $p=0.15$, Fig 3B), but both had a positive impact when compared to control *A. gossypii* populations with *H. convergens* present (*L. humile*: $F_{1,408}=5.15$, $p=0.023$, *T. sessile*: $F_{1,408}=5.18$, $p=0.0024$, Fig 3B).

Parasitism by *Aphidius colemani*

Over 50% of *A. gossypii* were parasitized by *A. colemani* when ants were absent (Fig 4). Parasitism was reduced similarly by both ant species (*L. humile*: $F_{1,18}=10.52$, $p=0.0031$, *T. sessile*: $F_{1,18}=6.29$, $p=0.018$, Fig 4), but was not significantly different between the two ants ($F_{1,18}=0.54$, $p=0.47$, Fig 4)

Field assessment of aphid protection by ants

Although there were more numbers of *L. humile* the first four days of the trial (Fig 5A), for the entire trial numbers were not significantly different ($F_{1,28}=0.28$, $p=0.60$). Mesh-covered plants had higher aphid numbers than all exposed plants ($F_{1,582}=9.33$, $p=0.0024$), suggesting that *A. gossypii* were affected by natural enemies in our field plot (Fig 5B). The supplemental diet with Tanglefoot® barrier did not affect predator abundance and *A. gossypii* populations were similar to those in exposed treatments with aphids and no ants ($F_{1,582}=0.34$, $p=0.56$, Fig 5B). There were more *A. gossypii* on plants with *L. humile* present than *T. sessile* ($F_{1,582}=3.71$, $p=0.054$, Fig 5B); however both ants did supply some protection to *A. gossypii* compared with the non-ant treatment (*L. humile*: $F_{1,582}=35.05$, $p<0.0001$, *T. sessile*: $F_{1,582}=15.95$, $p<0.0001$, Fig 5B). Predators observed most frequently on the plants were adult Coccinellidae, (including *H. convergens*, *Harmonia axyridis*, and *Coccinella sanguinea* var. *polita*). The same number of these beetles was recorded across all treatments ($F_{1,56}=0.63$, $p=0.60$) except those plants covered with mesh. Other predators observed included *C. carnea*, but there were too few and not enough of any other natural enemy group or guild observed to analyze ant impact of their abundance.

Discussion:

We demonstrated that an invasive and native ant had a similar impact on selected *A. gossypii* natural enemies, being particularly effective at reducing aphid predation by *C. carnea* and *H.*

convergens at low aphid numbers. When *C. carnea* larvae came into contact with the ants, the larvae had an immediate escape response, running quickly away from the foraging ant. The ant, alerted to the predator's presence, would pursue, but did not seem able to follow the exact trajectory of the predator's escape. *C. carnea* would remain stationary until probed again by the ant, and after a few minutes would either successfully evade the ant or be driven from the plant temporarily. No physical conflict was seen between either ant species and *C. carnea*. Other studies have shown that lacewing activity is reduced in the field when *Solenopsis invicta* is present (Kaplan and Eubanks, 2005; Eubanks, 2001; Eubanks et al. 2002) and predation of first stadium larvae has been recorded by *L. humile* (Grover et al. 2008), but the ant-predator interactions witnessed in this experiment have not, to our knowledge, been documented before. Likewise, with *H. convergens* adults exhibited similar escape responses as *C. carnea*, which would be deterred similarly and often take flight off the plant in response to the ant agitation.

Our observations are similar to avoidance experiments involving the ladybird beetle, *Adalia bipunctata* and *L. niger*, where the beetles avoided aphid colonies when ants were present and oviposited away from ant controlled territories (Oliver et al. 2008). *L. humile* and *T. sessile* also had similar effects on the oviposition ability of *A. colemani*. The parasitoid appeared to wait until ants were absent from a patch of aphids and fly in and quickly oviposit. Oviposition was very fast taking ca. 1-2 seconds; however there was almost always ants present in close proximity, reducing the opportunity for oviposition without ant

interference. The parasitoids seemed to sense ant presence before the ants were able to initiate contact and immediately retreated.

There are several examples of ants reducing hemipteran parasitism (e.g. Völkl, 1992, for reviews see Buckley, 1987a,b; Stadler and Dixon, 2005), including *L. humile*, (Martinez-Ferrer, 2003). There has also been much work looking at the mechanistic responses of parasitoids, e.g., *Pauesia* parasitoids to avoid antagonistic interaction with *Formica* ants tending various *Cinara* aphid species (Völkl, 1997, Völkl and Novak, 1997; Völkl and Kroupa, 1997). The behaviors observed in these systems include quick evasive movements and dropping behaviors by *Pauesia* wasps. There are several imperfections and exploitations parasitoids use to circumvent ant interference (Stadler and Dixon, 2005; Völkl, 2001; Kaneko, 2002, 2003), but in our laboratory experiments *A. colemani* was only partially able to circumvent antagonistic interaction by *L. humile* or *T. sessile*

Based on the available evidence that invasive ants can cause profound population density increases in honeydew-producing hemipterans (e.g. Abbott and Green 2007), presumably due in part to their aggression towards the natural enemies of these Hemiptera, we were somewhat surprised that *L. humile* and *T. sessile* performed comparably in our assays. In all experiments the number of *L. humile* on the plants was at least two-fold higher than that of *T. sessile*. Declines of *T. sessile*-tended *A. gossypii* at initial high densities following the introduction of *H. convergens* or *C. carnea* suggest that *T. sessile* are less able than *L. humile*

to protect dense aphid aggregations. Density dependent effects are evident in other ant-aphid-natural enemy communities. For example, the benefits to *Aphis varians* on fireweed from *Formica cinerea* attendance diminished as aphid density increased (Breton and Addicott, 1992). However, when ant number is limiting in ant-hemipteran interactions such as *F. altipetens* tending *Publilia modesta*, ant benefit is directly proportional to hemipteran population size (Cushman and Whitman, 1989).

That *T. sessile* mostly provides protection comparable to *L. humile* (at low aphid numbers) with ca. half as many foragers suggests that it actually protects aphids on a per ant basis better than *L. humile*, and that *L. humile* requires more foragers to effectively defend *A. gossypii*. Devoting a larger percentage of workers to a resource is key to an invasive ant's success because invading propagules are almost always numerically inferior to established native ants (Holway and Case, 2001; Hee et al., 2000), and increased foraging tempo may secure food to promote faster colony growth (Davidson, 1998; Davidson et al. 2003, 2004). Positive feedback between the invasive ant and honeydew-producing hemipteran results when elevated colony densities and foraging range expansion support higher hemipteran populations, which in turn fuel invasive ant population growth (Ness and Bronstein, 2004). This feedback mechanism is thoroughly illustrated in island ecosystems invaded by *A. gracilipes* which is responsible for damaging outbreaks of honeydew-producing Coccidae (Haines and Haines, 1978, O'Dowd et al 2003). Insecticidal control of *A. gracilipes* resulted in near elimination of the trophobiont scales (Abbott and Green, 2007). We provide

evidence that the native *T. sessile* is more effective protecting *A. gossypii* at lower vs higher population levels. *Lasius neoniger*, native to North America, also more effectively protected *Aphis fabae* from predation by adult coccinellidae at low aphid densities (Harmon and Andow, 2007).

Though loss of genetic diversity and reduced intraspecific aggression in introduced *L. humile* populations may contribute to colony expansion and displacement of native ants (Holway et al 1998, Tsutsui et al., 2000; Tsutsui and Suarez 2003), it is unlikely that disparate small introductions of *L. humile* would encounter each other and interact. Therefore, other mechanisms underlying invasion success require consideration. We favor superiority over native ants in locating and dominating resources, particularly high energy fuel such as hemipteran honeydew as a critical element for incipient colony expansion (Davidson, 1998; Human and Gordon 1996, 1999). We demonstrated, with an equal number of worker ants, that *L. humile* foragers exceeded those of *T. sessile* by at least 2-fold. Though this forager number disparity appeared unimportant in the relative protection afforded *A. gossypii* by these ants rapid acquisition of honeydew and its direct and indirect conversion to ant biomass may create numerical advantages for *L. humile* leading to resource and habitat.

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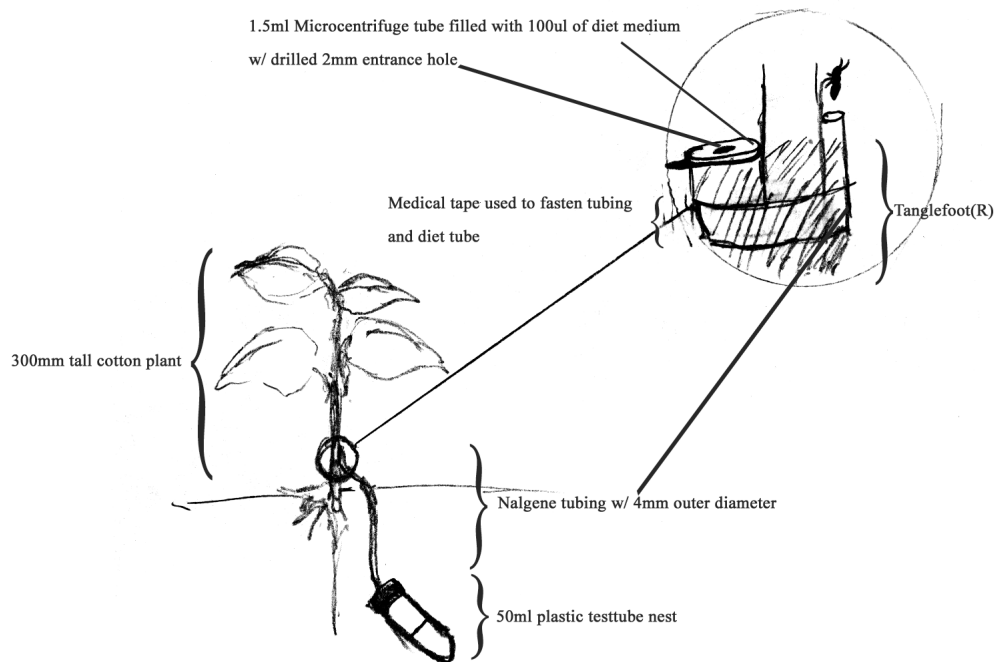


Figure 1: Schematic diagram of arrangement allowing *L. humile* or *T. sessile* access to *A. gossypii* in the field.

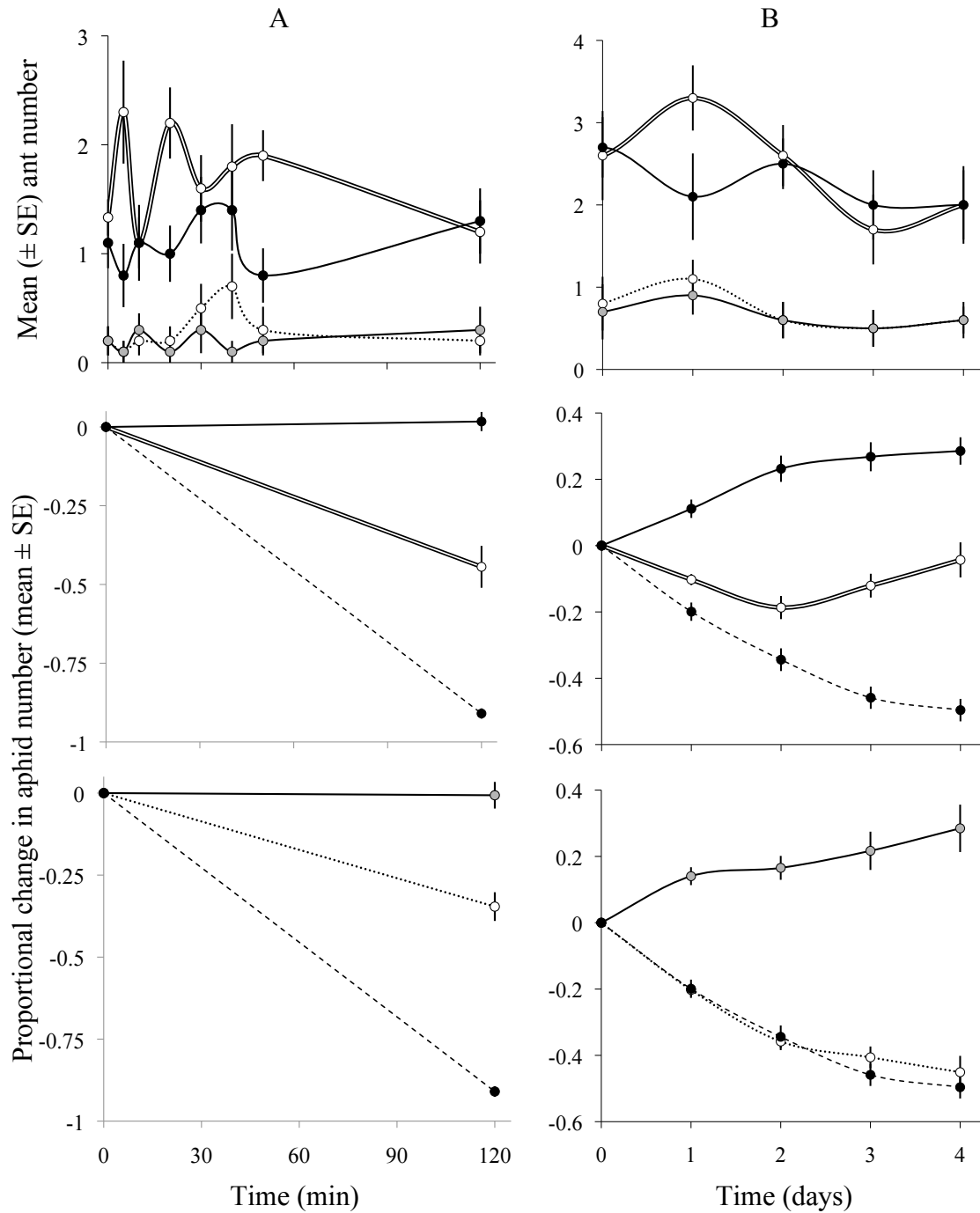


Figure 2: Mean (\pm SE) proportional change in aphid number and ant number during exposure to *C. carnea* larvae. Column “A” represents ants and ca. 100 *A. gossypii* exposed to *C. carnea* larvae. Column “B” represents ants and high population levels of *A. gossypii* exposed to *C. carnea*. $\cdots\circ\cdots$ *T. sessile* and *C. carnea* present, $\text{---}\circ\text{---}$ *T. sessile* present, $\text{---}\bullet\text{---}$ *C. carnea* present, $\text{---}\circ\text{---}$ *L. humile* and *C. carnea* present, $\text{---}\bullet\text{---}$ *L. humile* present.

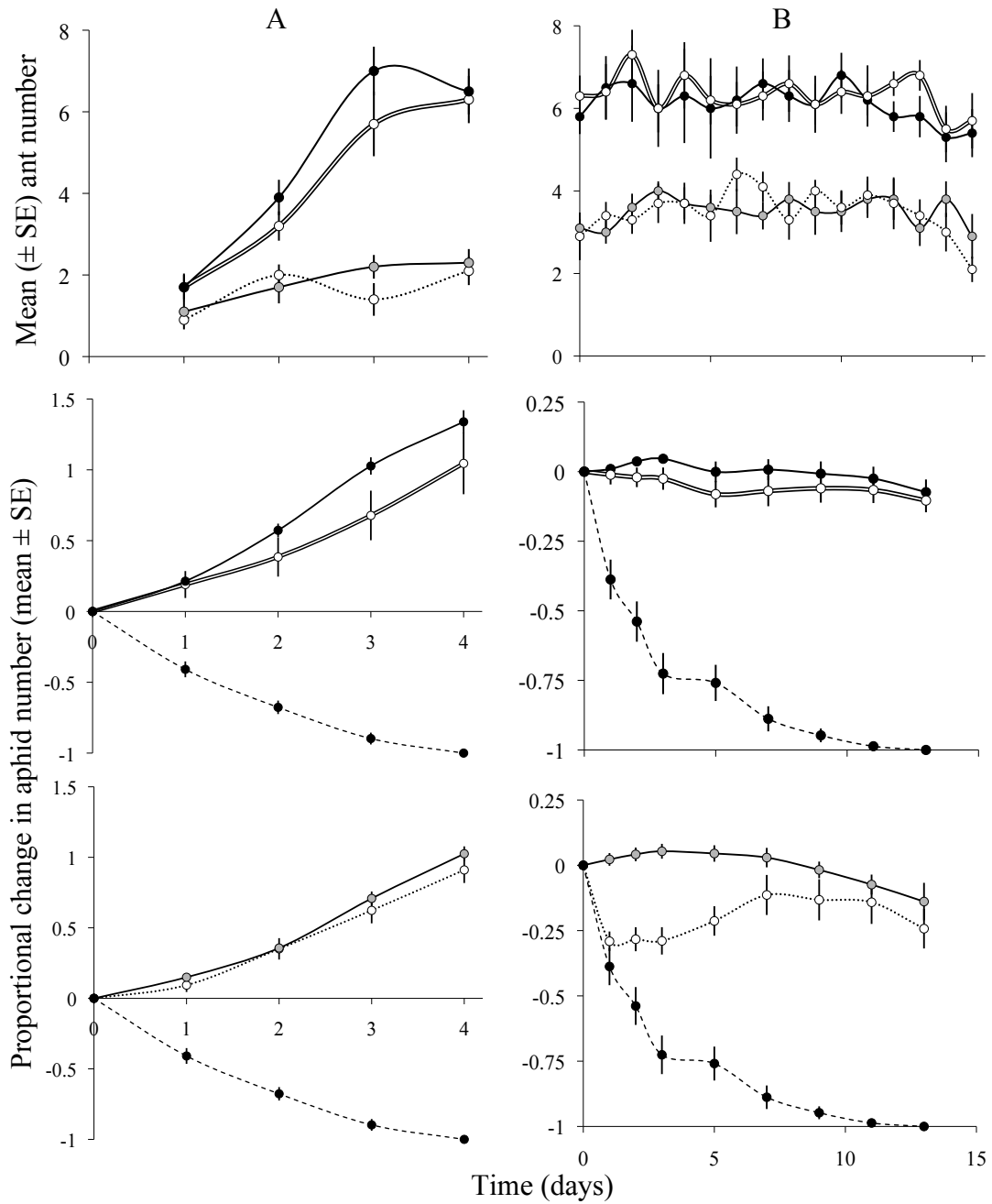


Figure 3: Mean (\pm SE) proportional change in aphid number and ant number during exposure to *H. convergens*. Column "A" represents ants and ca. 100 *A. gossypii* exposed to *H. convergens* adults. Column "B" represents ants and high population levels of *A. gossypii* exposed to *H. convergens* adults. $\bullet\text{---}\bullet$ *T. sessile* and *H. convergens* present, $\circ\text{---}\circ$ *T. sessile* present, $\ominus\text{---}\ominus$ *H. convergens* present, $\equiv\text{---}\equiv$ *L. humile* and *H. convergens* present, $\bullet\text{---}\bullet$ *L. humile* present.

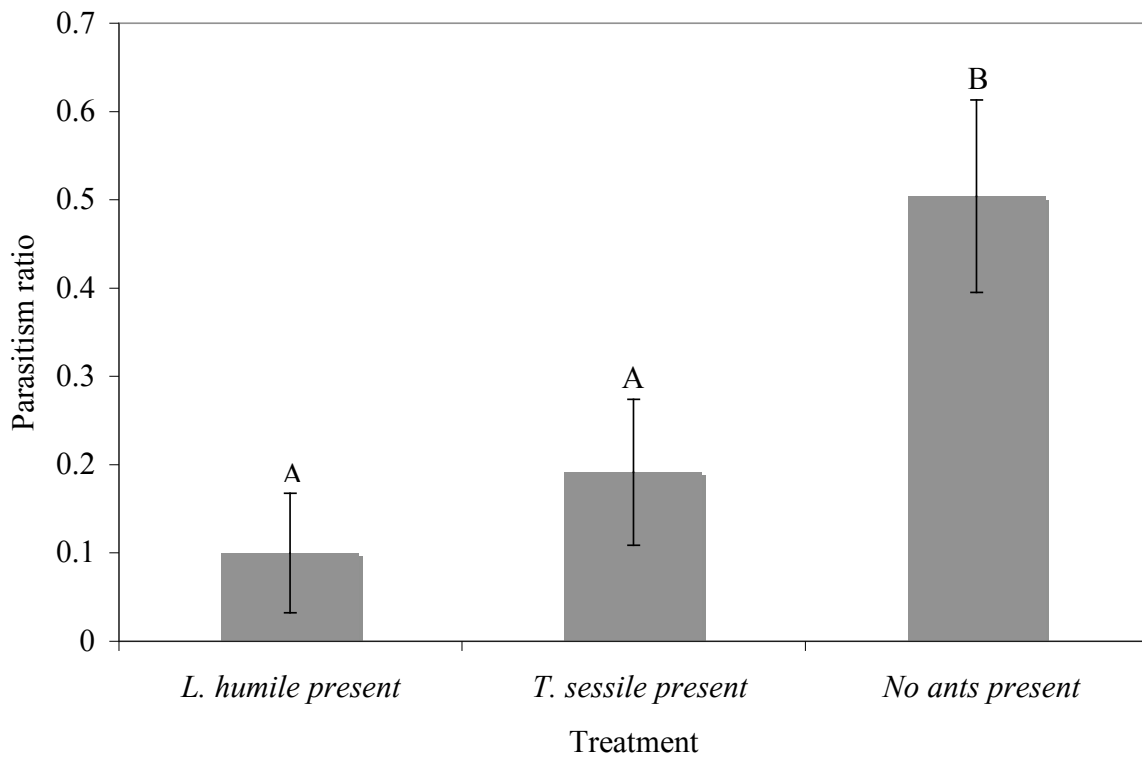


Figure 4: Effect of *L. humile* and *T. sessile* on parasitism of *A. gossypii* by *A. colemani*. Bars represent the mean (\pm SE) parasitism ratio (number of aphids parasitized/initial aphid number). Different letters indicate significant differences at 0.05 alpha level, data was arc-sine, square root transformed and analyzed using SNK.

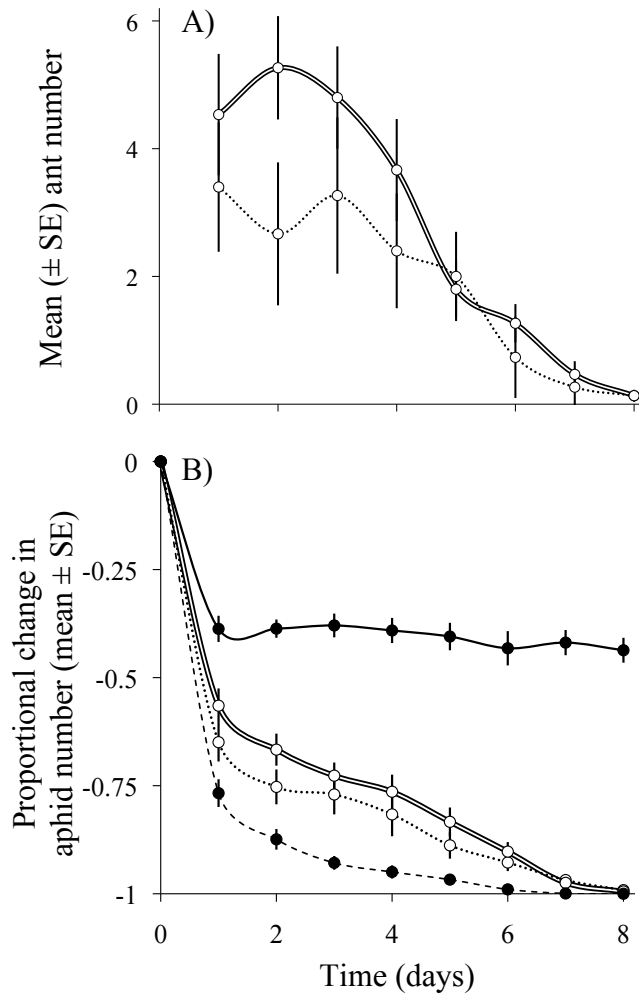


Figure 5: Effect of *L. humile* and *T. sessile* on *A. gossypii* field populations. $\bullet\text{--}\circ\text{--}\bullet$ *T. sessile* present, $\text{--}\circ\text{--}$ *L. humile* present, $\text{--}\bullet\text{--}$ No ants present, $\text{--}\bullet\text{--}$ No ants present, with viral mesh enclosure around all replicates. A) Mean (\pm SE) ant numbers per plant over time, B) Mean (\pm SE) proportional change in *A. gossypii* populations per plant over time,

Chapter 4: Competition between the ants *Linepithema humile* and *Tapinoma sessile* in the presence of *Aphis gossypii*

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Abstract:

The Argentine ant, *Linepithema humile*, has spread worldwide having severe effects within invaded natural, urban and agricultural ecosystems. *L. humile* can produce massive supercolonies spanning thousands of kilometers, but arrives into new territories in relatively small propagules. We investigated how introduced *L. humile* propagules might fare against established native ant colonies by recording the outcomes of competitive interactions of *L. humile* with *Tapinoma sessile*, an ant native to North America that co-occurs with or has been displaced by *L. humile*. We also determined the effect a honeydew-producing aphid, *Aphis gossypii*, had on these competitive interactions. With *A. gossypii* present *T. sessile* : *L. humile* worker ratios of 1: 1 and 2:1 resulted in a disproportionate amount of *T. sessile* displacement. *L. humile* was completely eliminated by *T. sessile* and consequently, unable to displace *T. sessile* at the 10: 1 ratio. Without aphids present on plants the ants showed significant reductions in aggression and were found coexisting at the same nest site. Without plants present (soil only) aggression was reduced and the ants avoided the nesting site. Our results demonstrate that *L. humile* does not need numerical superiority to displace a native ant from a nesting site and the aggression seems to be mediated by available carbohydrates. If carbohydrate resources are removed from an area *L. humile* may lose its competitive advantage, slowing invasion and promote native biodiversity restoration

Introduction:

Invasive species are one of the most influential factors in reshaping natural ecological systems (Elton 1958). While rarely beneficial (e.g. Brown 2003, Tecco et al., 2006), invasive species generally have a negative and often devastating impact on introduced ecosystems (Daehler, 2003; Dantonio and Vitousek, 1992; Franklin et al., 1999; Holway et al., 2002a; Liebhold et al., 1995). For example, Zebra mussels, *Dreissena polymorpha*, have had major impacts on freshwater ecosystems by reshaping community composition leading to overall losses in biomass and biodiversity of zooplankton (Ludyanski, 1993). *Phragmites australis* caused a shift in a marsh herbivore guild resulting in losses of nearly all predators and a breakdown in community structure (Gratton and Denno, 2005). Invasive ants are especially damaging to introduced environments, with the Argentine ant, *Linepithema humile*, (Formicidae: Dolicoederinae) is among the most destructive (Holway et al., 2002a). This species is capable of displacing local organisms from arthropods (Holway, 1998a) to lizards (Suarez and Case, 2002) and birds (Suarez et al., 2004; Peterson et al. 2004), with its greatest impact on local ants (Human and Gordon, 1996, 1999; Holway et al., 2002a). The success of *L. humile* is primarily attributed to its aggressive nature and ability to amass superior numbers to dominate resources (reviewed in Holway et al., 2002a). *L. humile* dominates much of its introduced range with supercolonies that may span thousands of kilometers (Tsutsui and Case, 2001; Giraud et al., 2002). Hee et al. (2000) argue that while *L. humile* colonies can reach huge sizes, they arrive into novel environments via smaller colony fragments or propagules. These authors demonstrated that *L. humile* propagules can

survive and expand with as few as 10 workers and one queen. These studies were conducted without subjecting *L. humile* to ecological barriers preventing establishment.

Biotic resistance from native ants could represent a formidable barrier to *L. humile* propagule growth and spread. Therefore, we investigated the survival of different sized *L. humile* propagules to an established native ant colony. *Tapinoma sessile* (Formicidae: Dolicoderinae) is a tramp ant (Passera 1994) native to North America with characteristics similar to *L. humile* (Buczkowski and Bennett 2006, 2008). Both ants can form large, polydomous, polygynous colonies and have a high affinity for hemipteran honeydew. Individual worker ants are approximately the same size and both ants occur within human-disturbed habitats (Buczkowski and Bennett 2006, 2008; Barbani, 2003; Smith, 1928). The distribution of both ant species overlaps in parts of the native range of *T. sessile* (Smith, 1965; Vásquez and Silverman, 2008). Since both ants utilize hemipteran honeydew and it has been proposed that *L. humile* colony expansion depends on this food resource (Rust et al., 2003; Holway et al. 2002), we investigated individual worker and colony level interactions in the presence of the honeydew-producing hemipteran, *Aphis gossypii* (Hemiptera: Aphididae) on extra-floral nectary (EFN) bearing cotton (*Gossypium hirsutum*). *A. gossypii* readily engages in facultative ant-mutualisms (Diaz et al., 2004; Kaplan and Eubanks, 2002; Vinson and Scarborough, 1989), including *L. humile* (Mondor et al., 2008) and *T. sessile* (BP personal observation). We varied *L. humile* propagule size to create discrete numbers of native : invasive worker ants, where *L. humile* would have inferior numbers to *T. sessile*.

This was used to investigate the notion that *L. humile* displaces native ants simply through superior numbers (e.g. Holway et al. 2002, Human and Gordon, 1996, 1999). Aggression in *T. sessile* and *L. humile* may be driven by nest location and not necessarily by the resources found at that location. By providing *T. sessile* with access to only soil and plants with no aphids in separate treatments, we investigated 1) the role of *A. gossypii* in affecting *T. sessile* nesting preference and 2) possible effects that the soil substrate and EFN bearing host plants had on aggressive behavior and nest site competition. The goals of this study were to understand the role of *L. humile* propagule size in invasion success and to identify possible mechanisms underlying competition for resources by *L. humile* and *T. sessile*.

Without adequate carbohydrate resources present at soil- only or uninfested plant control treatments we predict that *T. sessile* will not 1) establish a nest or 2) defend the aphid-less resource from invading *L. humile*. Similarly we do not expect *L. humile* to show a preference for colonizing a soil only resource if avoided or abandoned by *T. sessile*. There was a possibility that both ants would colonize the central container and thereby partition the resource. If resource partitioning occurred we predict that tending numbers would be proportional to the number of worker ants present by either species, otherwise the ant victor will maintain/assume complete control of the aphid/EFN resource. Since *L. humile* exhibits very high aggression to other ants (e.g. Rowles and O'Dowd, 2007, Holway, 1999), on an individual level, *L. humile* should outperform *T. sessile* and at the colony level, displacing *T. sessile* without needing a majority of ant workers.

Methods:

Organisms:

L. humile, *T. sessile* and *A. gossypii* were all maintained in an insect growth chamber (5.0m L x 2.8m W x 2.4m H) at 27C and 70%RH. Ants were collected from several discrete geographic locations in suburban North Carolina. Ants were kept in fluon lined containers supplied with 150mm petri dish nests filled with grooved moist plaster, water, 25% sucrose solution, artificial diet (Bhatkar & Whitcomb 1970) and freshly killed adult female cockroaches (*Blattella germanica*) ad libitum. *A. gossypii* were obtained from BASF Co. (Research Triangle Park, NC) where they were reared on cotton cotyledons, (*G. hirsutum*). Aphids were transferred every 10 days to fresh plant material.

Experimental design

We performed assays measuring interspecific aggression and nest site competition at *T. sessile* : *L. humile* worker ratios of 1:1, 2:1 and 10:1 in containers with plants and *A. gossypii*, plants only and soil only. Ten replicates per worker ratio and resource type were performed. These replicates were established consecutively due to the large amount of visual observation required. Each replicate consisted of three Fluon® lined plastic containers, two smaller containers (“A and B”, 250mm x 175mm x 40mm, Fig 1) were on either side of a larger central container (250mm x 380mm x 75mm, Fig 1). Both small containers had a Petri dish (75mm) filled with plaster, artificial diet, a test tube with water (25ml) and freshly killed female *B. germanica*. A pot of soil (150mm dia., Moisture Control Potting Mix, Scotts

Miracle Gro Company, Marysville, OH) with either 1) five ca. 300mm tall *G. hirsutum*, infested with *A. gossypii* (ca. 50aphids/cm²), 2) five ca. 300mm tall *G. hirsutum* without aphids or 3) no *G. hirsutum* was placed into the larger central container. A 300mm x 75mm corrugated cardboard bridge was placed between the small container A and the central container (Fig 1). We placed 1000 *T. sessile* workers and 10 queens into the smaller container A (Fig 1) and allowed *T. sessile* access to the central container for one week to provide adequate time to explore the central container and opportunity to relocate their nest. *Tapinoma sessile* commonly moves to a location where resources are present in the field as long as nest-substrate requirements are met (Buczowski and Bennett, 2006). We added the appropriate number of *L. humile* workers and queens to container B (Fig 1) depending on the desired *T. sessile*: *L. humile* ratio (i.e. 1:1 ratios received 1000 workers and 10 queens, 2:1 ratios received 500 workers and 5 queens and 10:1 ratios received 100 workers and 1 queen). After *T. sessile* was allowed to explore the cotton plant for a week, a cardboard bridge was placed between container B and the central container as described above (Fig 1). Both cardboard bridges were removed following 72 hours of continued *L. humile* exposure.

We recorded the outcome of individual interspecific worker interactions (i.e. *L. humile* killed or moribund, *T. sessile* killed or injured or both killed or injured) during the first 120 min.

We also recorded colony-level effects after 72 hours, with four possible outcomes, 1) only *T. sessile* or 2) *L. humile* present in the central container (1 colony), 3) both ants present in the central container (2 colonies), or 4) no ants present in the central container (0 colonies). We

counted the number of *T. sessile* on the cotton plants (where applicable) before and after *L. humile* introduction. Surviving *L. humile* and *T. sessile* (queens and workers) were counted after 72 h by collecting ants in moistened plaster nests after drying the soil.

Statistical analyses:

All statistical analyses were performed with SAS 9.1.3 (SAS Institute, 2002). *T. sessile* nesting preference prior to *L. humile* introduction (1=ants only detected in central container, 0.5=ants found in both central container and container A, 0=ants only in container A) was analyzed using a 3x3 Chi-square under PROC FREQ across resource treatments.

Interspecific colony interactions (0, 1 or 2 colonies in the central container post 72 hours of *L. humile* exposure pooled across *T. sessile*:*L. humile* ratios) as a function of resource (aphids present, plants with no aphids and soil only) in the central container was analyzed in a 3x3 chi-square with PROC FREQ. This analysis determined the primary outcome under each resource and treatments where one colony remained allowed for further analysis of competition using binomial or multinomial distributions against the following null hypotheses for each ratio under PROC FREQ. If worker ant number did not affect confrontation outcome than the expected win/loss percentage for either ant species would be 50%, this comparison was referred to as Null1. If neither ant had an intrinsic advantage over one another than we should expect that if *T. sessile* was in the majority (i.e. 1:10 and 1:2 ratios) they should have 100% victory over *L. humile* and in the 1:1 ratio we should expect a 50% win for either species; this comparison referred to as Null2. Logically, for the 1:1 ratio

Null2 is only valid since ant numbers were equal. Before individual ant aggression could be analyzed across resource treatments another 3x3 chi square was used to detect any advantages the ants had on the individual level as it related to the experimental ratio. If no trends were found then, individual ant confrontations were pooled, square-root transformed and analyzed across resource treatments using a PROC GLM ANOVA model, with SNK means groupings to assess the effect resources had on the level of individual ant aggression at each *T. Sessile:L. humile* ratio. Ant tending numbers (number of ants found on the plant pre and post *L. humile* exposure) were square-root transformed and analyzed using a 2-way ANOVA, with SNK means groupings. Proportional survivorship of ants, post 72 hours, was arcsine-square root transformed and analyzed across treatments using a PROC MIXED MANOVA model; treatment by ratio by caste combinations were contrasted with Tukey adjusted p-values.

Results:

Movement of T. sessile into central chamber nest:

The presence of plants had a strong affect on the movement of *T. sessile* colonies into the central container. Prior to introducing *L. humile*, most *T. sessile* colonies moved entirely from the peripheral plaster nest to within pots in the central container when the pots contained plants with or without *A. gossypii*, but fewer than one-half of the pots containing only soil had *T. sessile* ($X^2=50.9$, $p<0.0001$, Fig 2A)

Displacement of T. sessile colonies in central chamber by L. humile:

We recorded an overall effect of central container resource on *T. sessile* nest defense or displacement by *L. humile* ($X^2=90.0$, $p<0.0001$). The defense of the nest by *T. sessile* or its displacement by *L. humile* was largely mediated by the presence of *A. gossypii* (Fig 3A). Most nests were occupied by both *T. sessile* and *L. humile* with plants minus aphids (Fig 3B). Soil-only nest substrates were mostly abandoned (Fig 3C). *T. sessile* were never displaced by *L. humile* when aphids were absent. Since nest defense by *T. sessile* or displacement by *L. humile* was the primary response in the aphid treatments we analyzed the outcomes further using binomial distributions with the above described null models. A binomial distribution was used in this trial because no instances of cohabitation or abandonment were recorded. In the 10:1 *T. sessile* : *L. humile* trial: the number of ants (Null1 not supported) explained *T. sessile* defense of the central container ($p<0.001$), and no species effect, i.e. intrinsic fighting advantage by *L. humile*, was not found ($p=0.90$). In the 2:1 trial: ant number was not as important ($p=0.38$), while it seemed an intrinsic advantage by *L. humile* was present ($p<0.0001$), as it successfully displaced or triggered *T. sessile* to retreat in four replicates. Of these four replicates an average of 416.8 workers and 7.0 queens escaped to adjacent plaster nests. When numerically equivalent to *T. sessile*, *L. humile* extirpated *T. sessile* (no *T. sessile* recovered from these replicates) from the central containers eight out of 10 replicates, which was significantly more than expected ($p=0.05$).

Individual ant confrontation:

There was no effect of worker ratio on the number of aggressive interactions ($X^2=1.23$, $p=0.87$). Therefore we pooled our data and analyzed them across resource treatments. The highest level of aggression between *T. sessile* and *L. humile* occurred when *A. gossypii* was present (Table 1, Fig 2B). There was no difference in aggression levels between plants without aphids and soil only treatments (Table 1, Fig 2B).

Ant tending activity:

T. sessile showed a clear preference for foraging on plants w/ aphids vs. plants with only EFN present ($F_{1,58}=563.4$, $p<0.0001$, Fig 4A). Each ratio differed in the number of tending *T. sessile* lost due to *L. humile* exposure, with very little change at the 10:1 ratio (Fig 4B), with significantly more tending ants lost at the 2:1 ratio ($F_{1,18}=9.24$, $p=0.0052$, Fig 4B) and the most ants lost at the 1:1 ratio ($F_{1,18}=12.78$, $p=0.0013$, Fig 4B).

Ant survivorship:

All workers and queens in plant and soil treatments had similar levels of survivorship, with one exception of *L. humile* queen survivorship in the 10:1 ratio, soil treatment (Table 2, Fig 5). In that treatment-ratio combination every single *L. humile* queen was recovered, yielding no variance and leading to statistically higher survivorship. Survivorship was generally greater in soil and plant treatments except 1) at the 10:1 ratio, where *T. sessile* workers and queens had similar levels of survivorship (Table 2, Fig 5A) and 2) at the 2:1 ratio *T. sessile*

queens had higher survivorship (Table 2, Fig 5B). Aphid treatments were as follows per ratio: 10:1 and 2:1, *T. sessile* had greater survivorship than *L. humile* workers and queens (Table 2, Fig 5A, B, respectively), 1:1 both ants had low survivorship (Table 2, respectively, Fig 5C).

Discussion:

We determined that *L. humile* workers could effectively compete against *T. sessile* when outnumbered by as much as 2:1. We provide additional support for the competitive dominance of *L. humile* (Buczkowski and Bennett, 2008; Holway 1999; Human and Gordon, 1996; 1999); except our studies used discrete numbers of ants and *L. humile* was not in the majority. We also suggest that the invasive success of the Argentine ant is due, in part, to the resistance of small aggressive propagules to native ants. Interestingly, ant aggression increased in the presence of the aphid *A. gossypii* and both *T. sessile* and *L. humile* frequently shared nesting substrate in containers with plants, but not when aphids were on those plants.

T. sessile, at the individual level, was able to immobilize and kill just as efficiently as *L. humile*. Our work supports the findings of Buczkowski and Bennett (2008), where the percentage of winners in *L. humile*-*T. sessile* contests was not significantly different. This was particularly interesting considering the disproportionate number of *L. humile* victories at the colony level. Buczkowski and Bennett (2008) provided evidence that *L. humile* used group fighting strategies to suppress native ants. The results show that *L. humile* did not

need numerical superiority to disrupt a native system. In fact even at a 2:1 disadvantage, *L. humile* was able to usurp a resource from *T. sessile*. The data also suggest, that *T. sessile* colonies can only defend their territory against *L. humile* when they have a very large numerical advantage. When the numbers were equal, *L. humile* clearly had an advantage, but *T. sessile* colonies in two cases were able to defend the honeydew resource and the survivorship data showed significant losses by both ant species.

The fundamental difference between these two species may be the decision making process during the course of the confrontation. *L. humile* may devote more ant workers to overtake a common resource than *T. sessile* is willing to lose defending that resource. As Tanner (2006) explains, understanding the fighting strength (i.e. numerical status vs. competition) is essential for an organism's decision making process during a fight. This author demonstrated in another ant-ant interaction with *Formica xerophila* and *F. integroides*, that *F. xerophila* changed its aggressive behavior based on density cues prior to confrontation. Ants communicate information about colony size largely through chemical (pheromone) means (Hölldobler, and Wilson, 1990) and quite possibly this feedback is crucial during confrontations. Some systems such as those of competing colonies of *Myrmecocystus mimicus* the numerical strength of colonies can be ascertained from confrontation boundaries of the territory (Lumsden and Hölldobler, 1983); however, the communication pathway, e.g. chemical or visual is unclear. In our system, given the lack of obvious visual displays seen with *M. mimicus* (conspicuous posturing), there appeared to be a point of diminishing return

of worker victories possibly chemically signaled from the battlefield that triggered the decision to retreat. To our knowledge there has been no study that specifically investigated the effect of pheromone or other chemical cues in signaling retreat of ants from an attacker. There is evidence that trail pheromones can be used to signal retreat for *Lasius niger* from that same trail based on the timing and concentration of the pheromone (Kitabayashi and Gunji, 1997). It is possible that *T. sessile* uses similar cues to decide whether to defend or abandon a resource. *T. sessile* was only able to successfully retreat from the resource in the 2:1 confrontations, and was not able to retreat in any of the replicates during the 1:1 confrontations. Given that neither ant has an apparent advantage at the individual level, *T. sessile* either ceases to fight or *L. humile* is able to fight more effectively within the soil nest. Witnessing this behavior would be extremely difficult, if not impossible, but it is the only explanation as to why no live *T. sessile* were recovered from the 1:1 aphid trials.

Aggression for both species was mediated by the presence of *A. gossypii*. *T. sessile* clearly preferred nesting in soil where cotton plants were present either as a function of the permeability of the soil caused by its roots, the presence of EFN, or the combination of both. However, these factors alone did not induce aggression by *T. sessile* or *L. humile*. These results combined with our data on tending activity demonstrate that the EFN in this system do not provide a significant carbohydrate source. These findings are contrary to other ant-aphid interactions where EFN's can be a competitive resource compared with aphid honeydew (Sakata and Hashimoto, 2000). The reduced carbohydrates on non-infested plants

surprisingly allowed for cohabitation of both ants in the same nest site, with both species sequestering exudates from EFN on the same group of plants. Either of these ants tolerating an interspecific ant in the same nesting site is unprecedented. *T. sessile* only defended the soil when aphids were present, which seems in keeping with territorial theory as the energy required to defend an area should be less than the energy or resources provided by that area (Davies and Houston, 1984). Similarly, *L. humile* did not displace or extirpate *T. sessile* in any of the trials where aphids were absent, regardless of worker number.

There is conflicting evidence as to whether food availability alters aggression levels in *L. humile*. Thomas et al. (2005) reported that food did not influence intraspecific aggression, yet Grover et al. (2008) demonstrated that sucrose-deprived *L. humile* colonies become less aggressive. Our work supports the latter conclusion, where both *L. humile* and *T. sessile* exhibited reduced aggression in the absence of *A. gossypii* honeydew, to a level where they resided in the same nesting site. Given the pot dimensions used in our study, the two ants were nesting at a maximum of 150mm and we observed the two ant species clustering as close as 25mm within the pots.

The dear-enemy hypothesis describes the development of tolerance between species that are usually aggressive to each other (Wilson, 1975). The formation and maintenance of this tolerance is based on the principle that resources lost fighting over a common resource are much greater than resources acquired by sharing that resource (Wilson, 1975). Tolerance by

T. sessile and *L. humile* here is similar to other dear-enemy systems, such as *Cyphomyrmex rimosus* and *Wasmannia auropunctata*, (Grangier et al. 2007), but further experimentation is needed to see if our “passive” ants are hostile to other *L. humile* and *T. sessile* colonies.

With our laboratory studies we can begin to understand one advantage *L. humile* has over native ants such as *T. sessile* and the motivation of one native and one invasive species to defend or take control of valuable resources. In cases where *L. humile* workers were in the minority, their “aggressive” behavior put it at a disadvantage most of the time leading to complete colony death by *T. sessile*. Overt aggression by *L. humile* became favorable once the numbers of *L. humile* to *T. sessile* began to equalize. Once *L. humile* worker numbers were within 50% of *T. sessile* a disproportionate shift to *L. humile* victories began, primarily based on intrinsic aggression at the colony level. The sheer numbers of *L. humile* was not as consequential as how *L. humile* colonies allocate worker resource to different tasks (Roulston and Silverman, 2002). In the *L. humile* home range, where colonies are far smaller (Pederson et al., 2006), this worker allocation might be necessary to properly compete for resources and territory among equally aggressive species (LeBrun et al. 2007). Our findings of reduced aggression in the absence of hemipteran resources have two main implications: reduced invasive success and conservation. Depriving *L. humile* of hemipterans or other resources has been suggested as a method for control (Rust et. al, 2003) and may lead to promising results; in addition a reduced carbohydrate diet may facilitate reduced aggression and thereby inhibit colony expansion. There are many examples citing the vigorous overtake by *L.*

humile of carbohydrate based baits (e.g. Human and Gordon, 1996, 1999; Holway 1999; Walter and Mackay, 2005), thought to be a function of large, overwhelming numbers. Here we see the first evidence for *L. humile* competitive ability diminished through the restriction of a resource. Controlling *L. humile* directly through chemical means has proven extremely difficult and cannot be accomplished without a more comprehensive control scheme (reviewed by Silverman and Brightwell, 2008). Secondly with loss of Argentine ant competitive ability, local ants and other native biodiversity may be preserved and possibly even restored if carbohydrate resources are kept in check in an *L. humile* invaded area. Perhaps the key to abating *L. humile* expansion and native biota depression is curing the sugar addiction that fuels aggression and drives Argentine ant expansion.

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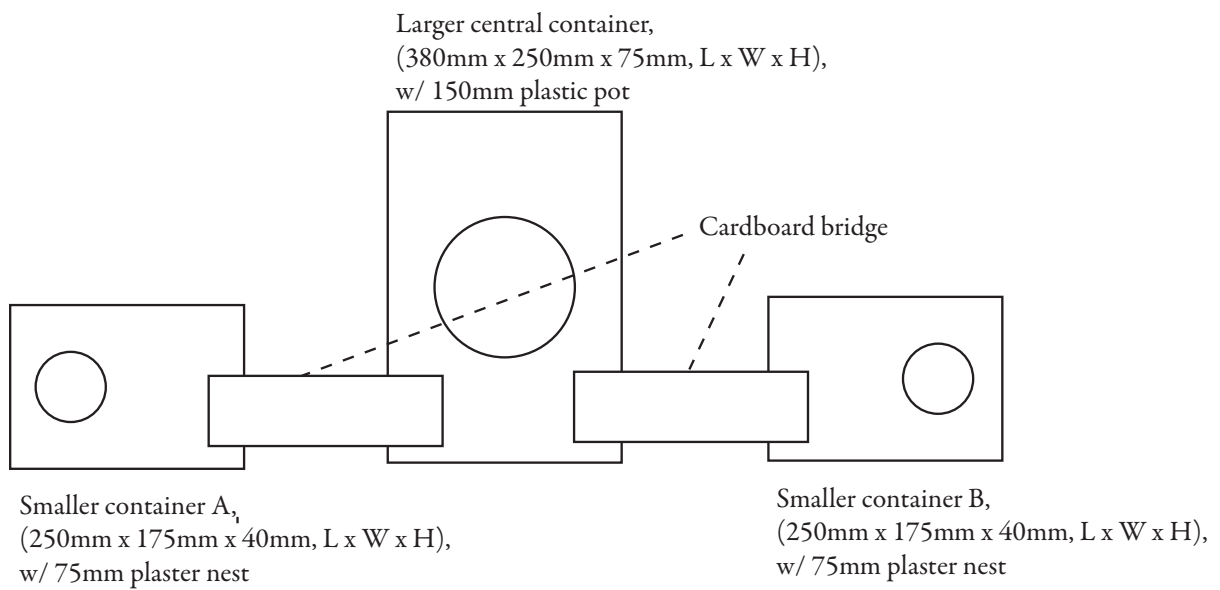


Figure 1: Top view schematic of the replicate setup

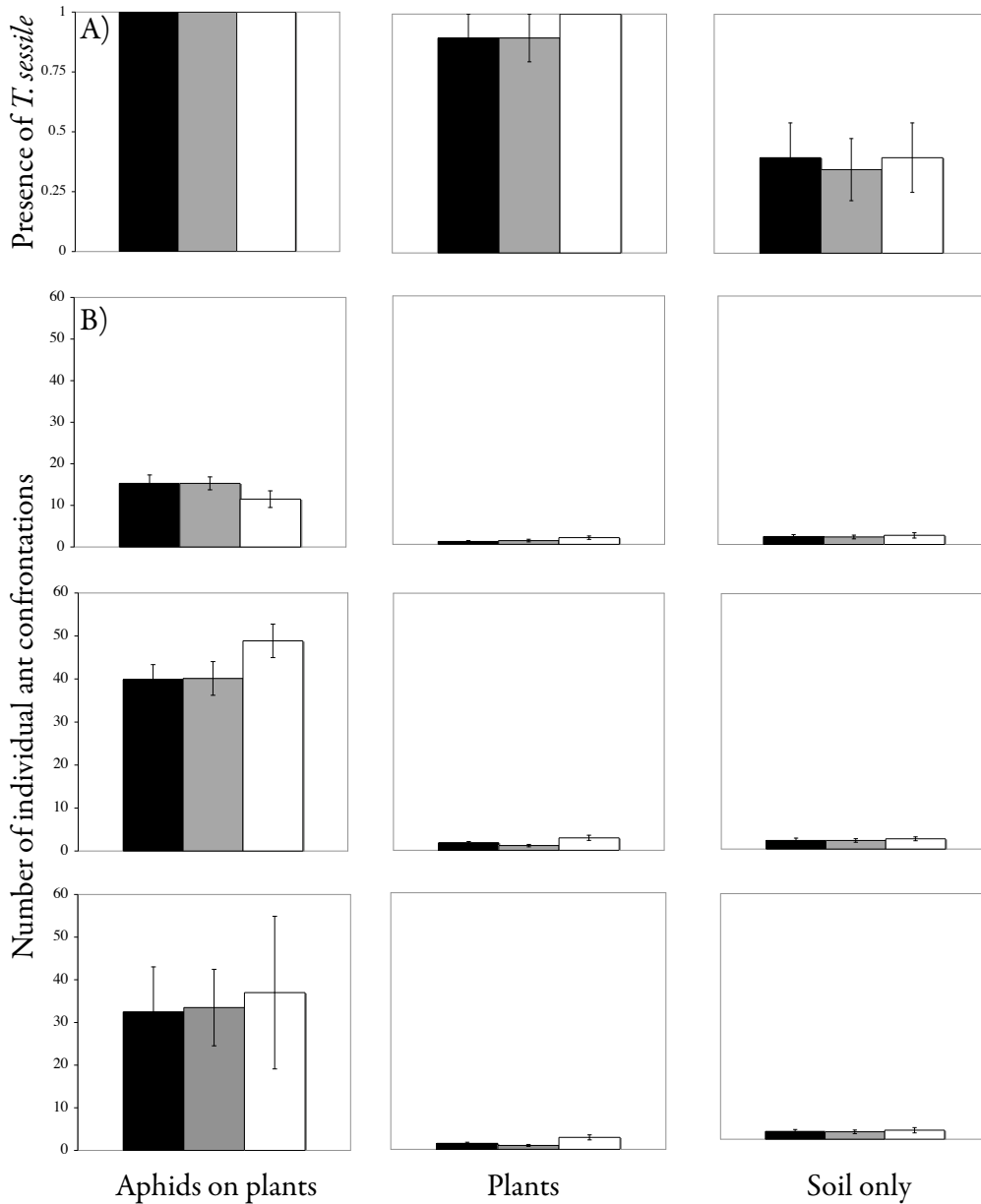


Figure 2: Presence of *T. sessile* in central container and individual ant confrontations across ratio and resource treatments. Columns (left to right) represent the central container w/ plants & aphids, plants only and soil only. A) Ratios of *T. sessile*:*L. humile*, ■ 1:1, ■ 2:1, □ 10:1. Mean (\pm SE) nesting preference of *T. sessile* 1=Central container 0=Plaster nest in adjacent container, B) Mean (\pm SE) Individual ant confrontations, ■ *T. sessile* win, ■ *L. humile* win, □ Draw. Top, middle and bottom rows represent 10:1, 2:1, 1:1 ratios of *T. sessile*:*L. humile*, respectively.

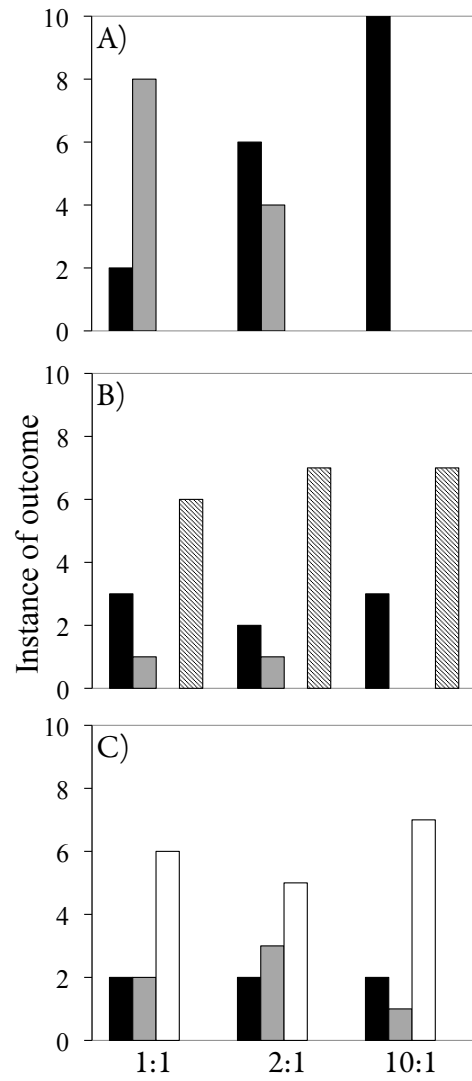


Figure 3. Colony confrontation outcome at the central container, ■ *T. sessile* win, ■ *L. humile* win, □ Abandoned, and ▨ Cohabitation. A) Aphids present, B) Plants with no aphids C) Soil only

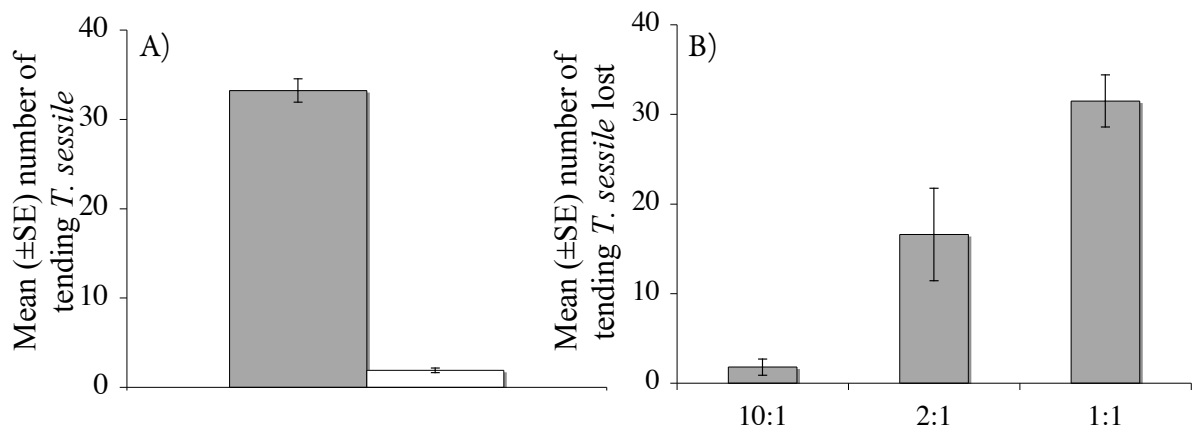


Figure 4. Tending activity of *T. sessile* before and after *L. humile* exposure. A) Mean (\pm SE) number of tending ants before *L. humile* exposure, Aphids present, Plants with no aphids present. B) Mean (\pm SE) number of tending *T. sessile* lost after 72 hours of *L. humile* exposure.

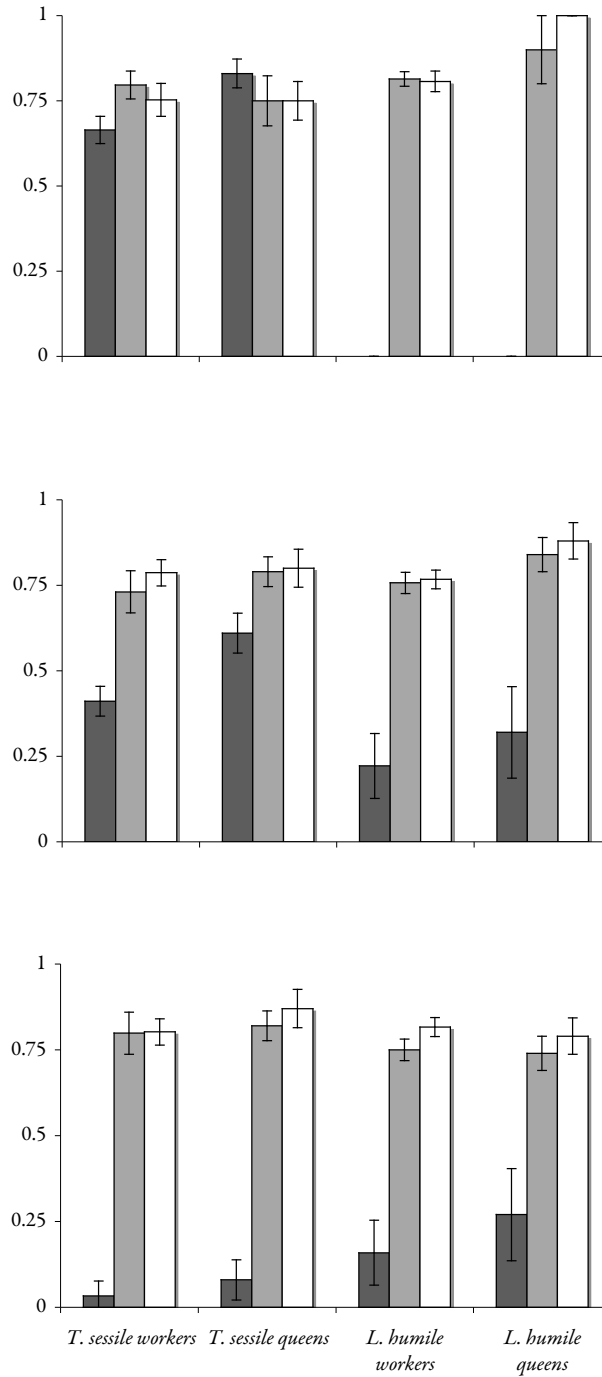


Figure 5. Mean (\pm SE) ratio of ant survivorship across ant ratios, \blacksquare Aphids present, \square Plants with no aphids, \square Soil only. A) 10:1, B) 2:1, C) 1:1 *T. sessile*:*L. humile* ratios

Table 1. Pooled individual ant aggression, PROC GLM ANOVA, w/ square-root transformation

Ratio - Treatment comparison	F-statistic	P-value
<i>10:1</i>		
Aphids vs. Plants	217.87	<0.0001
Aphids vs. Soil	196.16	<0.0001
Soil vs. Plant	0.57	0.46
<i>2:1</i>		
Aphids vs. Plants	27.81	<0.0001
Aphids vs. Soil	27.32	<0.0001
Soil vs. Plant	<0.01	0.96
<i>1:1</i>		
Aphids vs. Plants	4.52	0.043
Aphids vs. Soil	6.21	0.019
Soil vs. Plant	.13	0.72

Table 2: Survivorship significance table for *L. humile* and *T. sessile* workers and queens, (LhW, LhQ, TsW, TsQ, respectively). Capital letters are used for comparison across the rows and lower case letters are used comparison down columns. Different letters represent significance below the 0.05 alpha level and comparisons only valid within each ratio, PROC MIXED MANOVA, w/ Tukey adjustments.

10:1 ratio	TsW	TsQ	LhW	LhQ
Aphids	B a	B a	A a	A a
Plants only	A a	A a	A b	A b
Soil only	B a	B a	B b	A b
2:1 ratio				
Aphids	AB a	B a	A a	A a
Plants only	A b	A a	A b	A b
Soil only	A b	A a	A b	A b
1:1 ratio				
Aphids	A a	A a	A a	A a
Plants only	A b	A b	A b	A b
Soil only	A b	A b	A b	A b

Chapter 5: The impact of *Tapinoma sessile* and *Linepithema humile* on a field population of the aphid *Toxoptera aurantii*

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Abstract

Hemipteran honeydew is a valuable resource for many ants, including invasive species, where honeydew may fuel the extraordinary densities thought to contribute to their dominance within introduced ecosystems. Invasive ants are often associated with hemipteran outbreaks and therefore they may be better mutualists than native ant species. Yet, field studies comparing the impact of an invasive and native ant on a honeydew-producing hemipteran are lacking. We monitored numerical changes of the black citrus aphid, *Toxoptera aurantii*, tended by adjacent colonies of the invasive Argentine ant, *Linepithema humile*, and odorous house ant, *Tapinoma sessile*, native to North America on *Ilex sp.* Ant-tended aphid numbers were higher than those of untended aphids, with *L. humile*-tended and *T. sessile*-tended *T. aurantii* populations being comparable in 2005 and 2007. However, in 2006 aphid populations in areas occupied by *T. sessile* steeply declined during and immediately after a severe storm with heavy rainfall, suggesting that *T. sessile* tending behavior was negatively impacted by severe weather. A laboratory experiment simulating rainfall striking the surface of a leaf revealed that *T. sessile* foraging activity declined sharply under the most extreme circumstances whereas foraging activity of *L. humile* did not. We suggest that *L. humile* may gain a competitive advantage over ecologically similar native ant species by maintaining honeydew producing Hemiptera across a broader range of climatic conditions.

Introduction

Invasive species are one of the most influential factors in reshaping natural ecological systems (Elton 1958). In some cases invasive species have provided introduced areas with benefits, i.e. increased biodiversity, (Brown 2003, Sax and Gaines 2008). Invasive species, however, usually have negative, and in some cases, devastating impacts on introduced ecosystems, e.g. *Phragmites* and other plants (Gratton and Denno, 2005; Franklin et al., 1999) and zebra mussels (Ludyanski, 1993). Among the most destructive invasive taxa are ants, which are capable of displacing local organisms including arthropods (Holway, 1998a), reptiles (Suarez and Case, 2002), birds (Plentovich, 2008) and mammals (Allen et al., 2004). Invasive ants, such as the Argentine ant, *Linepithema humile* (Formicidae: Dolichoderinae), can disrupt communities, by displacing local ants (e.g. Carpintero et al., 2005; Holway et al., 2002; Erickson, 1971), due in large part to their aggressive behavior and competitive dominance (Holway et al., 2002). The success of *L. humile* is best demonstrated through their massive super colonies, in some cases spanning thousands of kilometers (Tsutsui et al., 2000; Giraud et al., 2002).

Studies of *L. humile* invasion boundaries have revealed a number of abiotic factors limiting boundary advancement, including elevation (Krushelnycky et al., 2005), temperature (Krushelnycky et al., 2005), soil composition (Bolger, 2007), landscape heterogeneity (Holway, 1998b; Holway et al., 2002) and, perhaps most important, proximity to water (Krushelnycky et al., 2005; DiGirolamo and Fox, 2006; Human et al., 1998; Holway et al.,

2002; Menke and Holway, 2006). While there is much support for abiotic factors affecting *L. humile* spread, there is little evidence for biotic resistance against *L. humile* establishment in new areas (Holway et al., 2002, but see Walters and Mackay, 2005). Native biotic resistance is primarily thought to be interspecific conflict with other ant species and competition for natural resources (Holway et al., 2002; Lebrun et al. 2007). There are a number of studies demonstrating the ability of *L. humile* to outcompete native ants at artificial bait stations (Holway, 1999; Rowles and O'Dowd, 2007; Human and Gordon, 1999). The Argentine ant has often been implicated in explosive outbreaks of honeydew producing Hemiptera (reviewed in Holway et al. 2002; Ness and Bronstein, 2004), and it is thought that a plentiful carbohydrate resource, such as honeydew, can fuel the high worker densities seen in *L. humile* infestations leading to local ant extinction (Davidson, 1997; Davidson et al., 2004; Ness and Bronstein, 2004; Holway et al 2002). There are few studies, however, demonstrating natural resource utilization between sympatric native ants and *L. humile*, which is perhaps not surprising given the propensity of invasive *L. humile* to displace most native ant species. By comparing honeydew resource utilization between *L. humile* and sympatric native ants we may gain insights into one possible mechanism (more effective mutualists) underlying the invasion success of the Argentine ant.

We discovered a unique site in Raleigh, North Carolina supporting both *L. humile* and *Tapinoma sessile* (Formicidae: Dolichoderinae) an ant native to North America. *T. sessile* is similar to *L. humile* in size and crop capacity and it readily utilizes hemipteran honeydew. Its

colony and population structure largely resembles that of *L. humile* being both polydomous and polygynous (Buczowski and Bennett, 2006, 2008a,b; Barbani, 2003; Passera, 1994). Like *L. humile*, *T. sessile* can become a significant urban pest (Barbani 2003; Buczowski and Bennett, 2006, 2008a; Smith, 1928). Both ants at our site were found tending the black citrus aphid, *Toxoptera aurantii* (Hemiptera: Aphididae), a facultative myrmeophile, which excretes large volumes of honeydew (Dartigues, 1992).

In general, aphids and other honeydew-producing hemipterans benefit from ant attendance (Bishop and Bristow, 2001; Buckley, 1987, Karhu, 1998; Way, 1963; Cushman and Whitman, 1989; Fischer et al., 2001, but see Stadler and Dixon, 1998), particularly through the deterrence of their natural enemies (Breton and Addicott, 1992; Fischer et al., 2001; Harmon and Andow, 2007). However, the relative performance of ant mutualists is poorly understood. Herein, we compare the survival and growth of a field population of *T. aurantii* when tended by *L. humile* or *T. sessile* and the impact of these ants on the local predators of *T. aurantii*. We predicted that *L. humile* would more effectively reduce predation on *T. aurantii* than *T. sessile* thereby facilitating higher aphid population growth.

While we have some understanding of the influence of the abiotic environment on *L. humile* distribution, virtually nothing is known about *T. sessile*. While tracking *T. aurantii* populations and ant foraging activity across three years we suspected that significant rainfall affected *L. humile* and *T. sessile* foraging activity differently leading to different consequences

for *T. aurantii*. Therefore, we simulated the effect of rainfall disturbance on *L. humile* and *T. sessile* foraging activity in the laboratory. Based on our field recordings we predicted that *L. humile* foragers would be less disturbed by heavy rainfall than *T. sessile* foragers.

Methods and materials:

We conducted our experiment within a hedge of Chinese holly (*Ilex cornuta*) in a commercial park (ca. 100 hectares) in Research Triangle Park, North Carolina from 2005-2007.

While *I. cornuta* hedges were abundant throughout the business park, both *L. humile* and *T. sessile* only occurred with *T. aurantii* on this plant within one small area, ca. 30 m² area split between both ant species. The boundary between the colonies of *L. humile* and *T. sessile* foraging on *I. cornuta* was discrete with no apparent interactions between the ant species. Aggregations of *T. aurantii* occurred only on new growth of *I. cornuta* 10-20 day windows in the summer of each year.

For each of the three years this system was studied, we haphazardly chose 30-40 *I. cornuta* branches with *T. aurantii* within each area occupied by *L. humile* or *T. sessile*. At least one ant tending *T. aurantii* was on the selected branch at the time of selection. Branches were identified with flagging tape (ENVC, Sherman, TX). We saw no evidence that the flagging tape affected ant foraging. We randomly selected one half of the *Ilex* stems and applied a ca. 50 mm wide Tanglefoot® barrier (The Tanglefoot Company, Grand Rapids, MI) below the

new growth. These served as ant-exclusion controls. We made daily checks to ensure that the Tanglefoot® barrier was intact, and it was reapplied if necessary.

We counted aphids each day, noted the presence of ants (2005 and 2006) and counted the number of ants tending aphids in 2007. We counted and identified predators of *T. aurantii* on each branch to the family level. However, due to the low number of each predator species, we pooled all predator species counts in 2005 and in 2006. The number of predators recorded was too low in 2007 to analyse statistically. Our discovery of this unique site with both *L. humile* and *T. sessile* in 2005 was quite fortuitous and, consequently we were not prepared to record the numbers of ants tending *T. aurantii* on each branch. In 2006, few *T. sessile* occurred on the plant following heavy rainfall (data from NOAA, 2008 reported) four days after our experiment began, thereby precluding a comparison of ant forager numbers.

Following the three years of field recordings, we tested our hypothesis that heavy rainfall was responsible for the decline of foraging *T. sessile* in 2006. We developed a laboratory assay to simulate the responses of *L. humile* and *T. sessile* to a rainfall event when a carbohydrate resource, such as aphid honeydew, was being sequestered. We used a 250 ml burette combined with various flow regulating devices, (Fig 1), to provide low (0.175 ml/sec), medium (0.283 ml/sec) and high (0.55 ml/sec) water release with a mean droplet size of 0.10 ml at all flow rates. Strawberry jam dabbed onto the underside of a flexible strip of plastic simulated honeydew from *T. aurantii* attached to on the underside of a leaf (Fig 1). Prior

laboratory work (Powell and Silverman, submitted), revealed that *L. humile* used, on average, proportionally more workers than *T. sessile* when tending the aphid *Aphis gossypii*. Therefore, a larger number of *T. sessile* workers (300 plus 3 queens) than *L. humile* (200 plus 2 queens) was used per replicate to ensure comparable numbers of foraging ants for both species. The ants were placed onto a 355 ml plastic cup containing moistened soil and a vertical wooden dowel [300mm (H) x 19mm (dia.)] with the jam daubed flexible plastic strip (150mm x 13mm) stapled to the end of the dowel. The cup was then housed within a Fluon-coated plastic container (40mm x 175mm x 250mm). Each replicate was supplemented with 2-3 freshly killed female German cockroaches (*Blattella germanica*) and deprived of any sugar source for 72 hours.

To ensure consistent vibration from the plastic strip when struck by a water drop, wooden shims were employed to make certain the plastic strips were horizontal after positioning under the burette. Care was taken to make sure that only the distal 10 mm of plastic strip was exposed to the water droplets, otherwise the water would displace the ants and jam from the plastic strip. Ten droplets of jam (total = ca. 0.39 mg) were then placed in a circular pattern ca. 15mm away from the distal end of the plastic strip. The time to discover the jam and initial recruitment were highly variable between replicates regardless of ant species, so we waited until there was no further recruitment to the sugar source for 30 sec before releasing water droplets. Ten replicates were performed for each of the three flow rates. Ants on the jam were counted just before and immediately after (< 10 sec.) droplets were released

followed by counts each minute for 10 min of continued water disturbance. We recorded only those ants on the jam. Control units were identical to the treatments except water was not applied.

Statistical Analysis

We analyzed both aphid population growth rate (number of aphids over time) each year, and ant numbers in 2007 among treatments and controls using a repeated measures models (PROC MIXED), with appropriate contrasts using SAS 9.1.3 (SAS Institute. 2002). The presence of ants (2005 & 2006) and predator counts were analyzed using a logistic data, prepeated measures model under PROC GENMOD with appropriate contrasts. The change in the number of ants during the simulated rainfall trials was analyzed with a repeated measures model (PROC MIXED) following log transformations of the proportional difference from the original recruited ant numbers.

Results

Aphid population changes

Across all years there was an eventual decline in aphid numbers due to the maturation of the *I. cornuta* stems, presumably rendering them unpalatable to *T. aurantii*. In 2005, there was no effect of either *L. humile* or *T. sessile* on *T. aurantii* population levels. The decline in aphid numbers was similar between ant species ($F_{1,472}=1.27$, $p=0.26$, Fig 2). Aphid popula-

tions tended by *T. sessile* and *L. humile* were no different from ant excluded controls ($F_{1,472}=1.61$, $p=0.21$, $F_{1,472}=1.27$, $p=0.17$, respectively, Fig 2).

In 2006, *L. humile* had a positive effect on *T. aurantii* survival while *T. sessile* did not. ($F_{1,832}=11.55$, $p<0.001$, Fig 2). Aphid populations changed little during the experiment when tended by *L. humile* compared with those on ant-excluded branches ($F_{1,832}=7.11$, $p=0.0078$, Fig 2). Aphid numbers declined both on branches where *T. sessile* had access and *T. sessile* excluded branches ($F_{1,832}=0.31$, $p=0.58$, Fig 2). Aphid populations tended by ants were lower on average in 2006 than in 2005 ($F_{1,82}=3.77$, $p=0.05$; Fig 3). There was a large amount of rain in 2006 that began ca. 4 days into the experiment and continued throughout, with several inches recorded, NOAA, 2008 (Fig 2).

In 2007, the decline in *T. aurantii* numbers through time was less when tended by either *L. humile* ($F_{1,712}=15.72$, $p<0.0001$, Fig 2) or *T. sessile* ($F_{1,712}=22.71$, $p<0.0001$, Fig 2) than when no ants were in attendance, but the affect on aphid numbers by both ants was similar ($F_{1,712}=0.12$, $p=0.73$, Fig 2). Overall, aphid populations were low compared to 2005 ($F_{1,82}=13.17$, $p = 0.0005$; Fig 3) and were similar to 2006 ($F_{1,82}=0.01$, $p =0.90$; Fig 3).

Ant and predator presence on branches with T. aurantii

There was no difference between *L. humile* and *T. sessile* in the number of branches containing workers in 2005 ($\chi^2 < 0.01$, $p = 0.96$; Fig 4), however, in 2006 fewer branches contained *T. sessile* than *L. humile* ($\chi^2 = 3.96$, $p < 0.05$; Fig 4). The number of *L. humile* and *T. sessile* per branch was, again, similar in 2007 ($F_{1,28} = 0.01$, $p = 0.92$; Fig 4). Syrphidae larvae and Coccinellidae larvae and adults were the most common predators found on *I. cornuta* branches. There was no difference in the total number of these predators regardless of the four ant treatments in 2005 or 2006 (Table 2, Fig 5). There were not enough predators recorded in 2007 to conduct any analysis.

Ant response to simulated rainfall

The response to the water agitation by *L. humile* and *T. sessile* was different at low ($F_{1,784} = 5.01$, $p < 0.05$), medium ($F_{1,784} = 31.90$, $p < 0.0001$) and high ($F_{1,784} = 38.25$, $p < 0.0001$) flow rates in our simulated rainfall experiment. *L. humile* moved rapidly towards water droplets at the end of the plastic strip, independent of flow rate. *L. humile* workers differed from undisturbed controls at medium ($F_{1,784} = 19.02$, $p < 0.0001$) and high ($F_{1,784} = 7.19$, $p = 0.0075$) flow rates by retreating from the carbohydrate resource initially but returning over the next several minutes. *L. humile* only exhibited this behavior marginally at the low flow rate ($F_{1,784} = 3.32$, $p = 0.07$, Fig 6). In contrast, the percentage of *T. sessile*, at the resource,

gradually declined over time during the medium ($F_{1,784}=3.98$, $p=0.046$) and high ($F_{1,784}=9.46$, $p=0.0022$) rates, but not during the low flow rate ($F_{1,784}=0.03$, $p=0.86$, Fig 6).

Discussion

Though field populations of *T. aurantii* on *I. cornuta* varied from 2005 through 2007 a couple of patterns emerged from our experiments. First, the impact of both *L. humile* and *T. sessile* on *T. aurantii* in 2007, but not 2005, may be proportional to aphid population size. Second, *T. aurantii* declines in 2006 within the territory of *T. sessile*, but not that of *L. humile*, may be related to a heavy rainfall event, which is supported by results from our laboratory experiment.

Both *L. humile* and *T. sessile* may only offer significant benefits at relatively low *T. aurantii* densities. *Lasius neoniger* was most effective at protecting *Aphis fabae* against predation by coccinellid adults when aphid numbers were low (Harmon and Andow, 2007) and *Formica cinerea* only provided benefits to smaller, initial populations of *Aphis varians* on fireweed, *Epilobium angustifolium* (Breton and Addicott, 1992).

The steep decline in aphid numbers in 2006 within the *T. sessile* territory but not in the territory patrolled by *L. humile* during a period of heavy rainfall combined with our laboratory results demonstrating that *T. sessile*, but not *L. humile*, vacated an area disturbed by water droplets suggest that *L. humile* remained with *T. aurantii* during this rainfall event while *T.*

sessile did not. Many aphid species will drop off host plants when disturbed (Dixon, 1986), including the brown citrus aphid, *T. citricida* (Michaud and Belliure, 2001). Ant-aphid mutualisms impose costs on aphids, which includes dispersal restriction (Stadler and Dixon, 1998; Stadler and Dixon, 1999), and *L. humile* may have prevented *T. aurantii* from dropping from the plant during the heavy rainfall. Although ants can move hemipterans to optimal feeding locations (e.g. Richerson and Jones, 1982, Stout, 1979), we provide the first indication that an ant may shield its hemipteran partner from being dislodged from its host plant due to rainfall. *L. humile* is native to floodplain woodlands in northern Argentina (Le Brun et al., 2007) and thus may be adapted to cope with extreme rainfall events. Rainfall can mediate ant behaviors, including nest building (Maschwitz and Moog 2000, Waldkircher and Maschwitz 2003) and nuptial flights (Kenne and Dejean, 1998). By preventing aphid dispersal and thus having honeydew available during and shortly after a heavy rainfall, *L. humile* may acquire carbohydrate fuel over longer periods than *T. sessile* and perhaps other hemipteran-tending native ants, which could contribute to the Argentine ant's dominance over native ants through the maintenance of more workers.

There were too few predators at the study site in 2007 for an analysis and in 2005 and 2006 we did not find more predators on ant-excluded vs. on branches where ant movement was unimpeded. Yet, aphid numbers declined in 2006 on branches where *L. humile* was excluded and controls in 2007 where both *L. humile* and *T. sessile* were excluded. We suspect that unattended aphids were preyed upon outside the period when data were

recorded, particularly at night. Greater predation of aphids by coccinellid adults and larvae has been recorded at night versus during the day (Vickerman and Sunderland, 1975) and there is some evidence syrphids have increased activity at night (Stubbs and Ball, 2002; Vickerman and Sunderland, 1975). Nocturnal feeding may explain our inability to detect a difference in Coccinellidae and Syrphidae larvae in ant excluded and unexcluded branches. The syrphid larvae may also evade detection by the ants (Way, 1963), possibly through chemical camouflage (Lohman et al., 2006).

Invasive ants may facilitate pest hemipteran outbreaks by displacing natural enemies, with hemipteran honeydew fueling the colony growth required to displace native ant fauna (Ness and Bronstein 2004, others). *L. humile* may feed for longer periods and develop larger colonies themselves. *S. invicta* colonies that had access to mealybug exudates vs. restricted colonies grew ca. 50% larger (Helms and Vinson, 2008). However, incipient colonies of invasive ants may provide no better services to the partner hemipterans (e.g. natural enemy defense) than native ants under optimal conditions (but see Ness and Bronstein, 2004). By remaining with *T. aurantii*, and perhaps other hemipterans, on leaf surfaces during severe climatic events that deter native ants, *L. humile* may have prevented the hemipterans from dropping to the ground and being lost. Alternatively, *L. humile* may be capable of continued feeding through climatic conditions that would deter some native ant species.

We have demonstrated that some climatic events such as rainfall may temporarily deter attendance of aphids by native ant species but not invasive ant species like *L. humile*. This differential attendance may have profound consequences on populations of myrmecophilic Hemiptera as suggested for *T. aurantii*. The maintenance of high numbers of honeydew-producing hemipterans by *L. humile* may lead to the dominance of the invasive ant over native ant species through the ability to maintain and fuel greater numbers of workers. We propose that this ability to better “conserve” honeydew-producing hemipterans from adverse climatic conditions may be one mechanism whereby an invasive ant, such as *L. humile*, can ultimately come to dominate an ant community.

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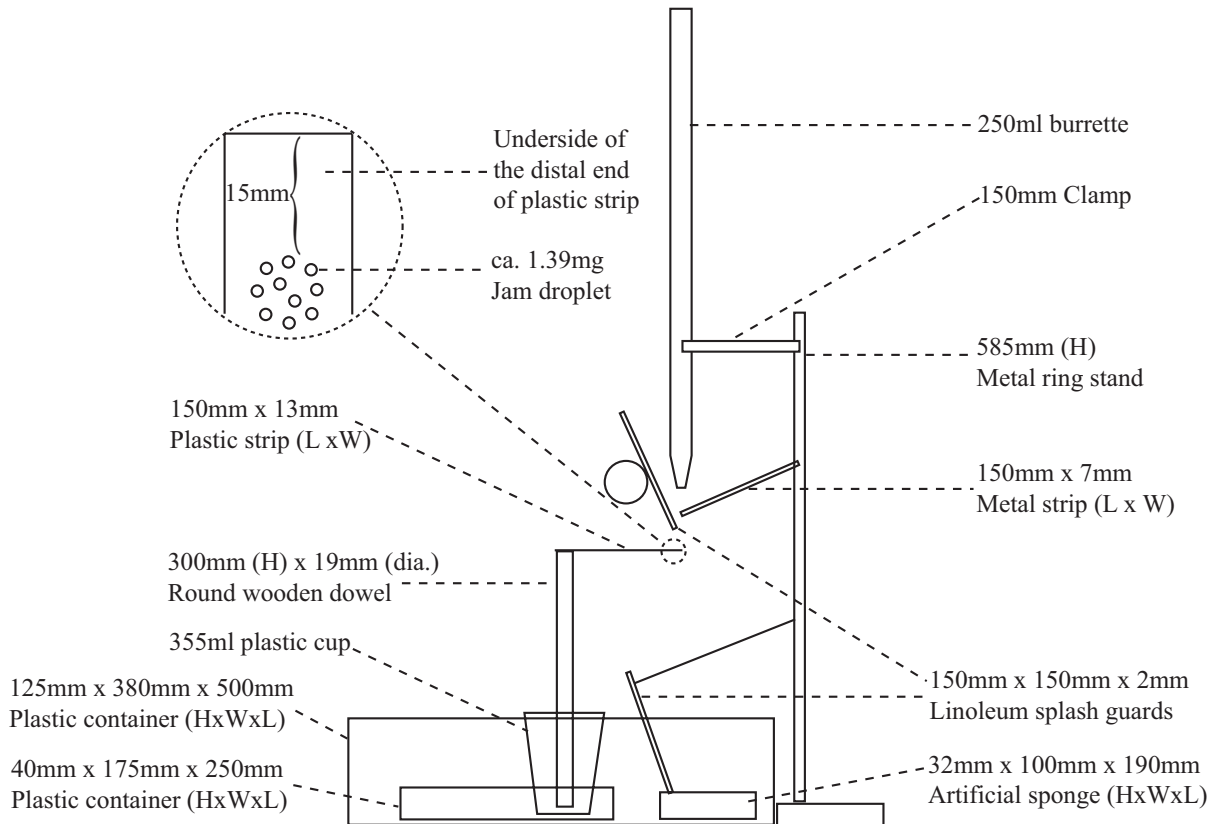


Figure 1. Schematic of the simulated rainfall apparatus drawn to scale except for the underside of the plastic strip insert. The linoleum splash guards were held at ca. 65.4° angle, the metal strip was held at ca. 23.6° angle. The lower splash guard was fixed to the ring stand via flexible tape on one side so that it could be moved out of the larger container to wring out the sponge between replicates. The tip of the water exit (i.e. burette or pipette tip) was positioned 15mm above the metal strip. The metal strip standardized droplet size, the splash guards kept water from physically removing the jam and or ants (upper) and water from entering the smaller container (lower). The flow adjustment knob on the burette was sufficient control for the high and medium rate but a pipette tip was affixed to the end of the burette to slow the flow for the lower rate.

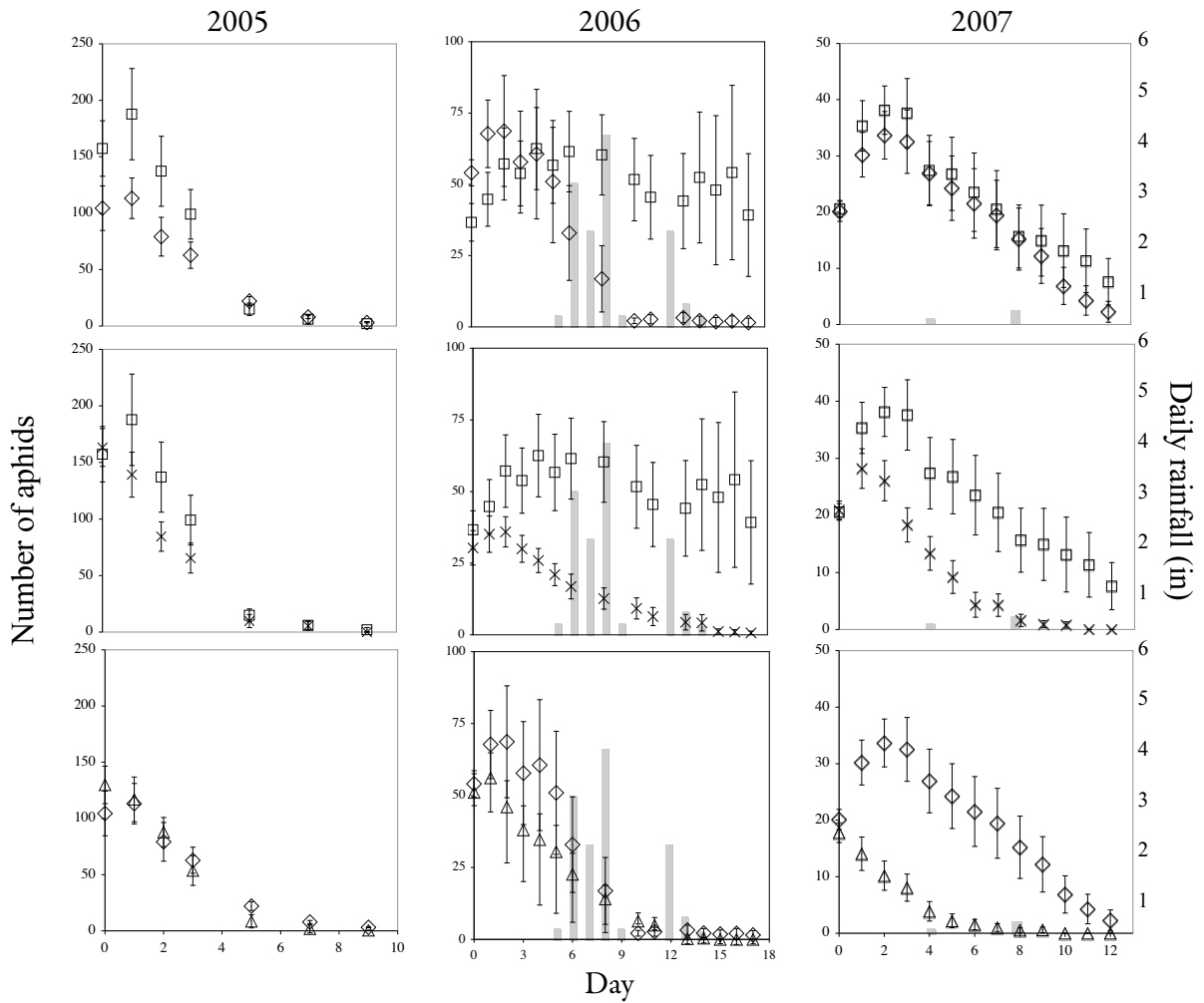


Figure 2. Daily rainfall and mean (\pm SE) *T. aurantii* numbers per tip through time for 2005-2007, \square *L. humile* present, \diamond *T. sessile* present, \times *L. humile* exclusion, \triangle *T. sessile* exclusion. Columns represent years of data, left to right, 2005, 2006 and 2007, respectively. The top row represents aphid populations in the presence of *T. sessile* and *L. humile*. The middle row represents aphid populations in the presence of *L. humile* and respective ant-exclusion controls. The bottom row represents aphid populations in the presence of *T. sessile* and respective ant-exclusion controls.

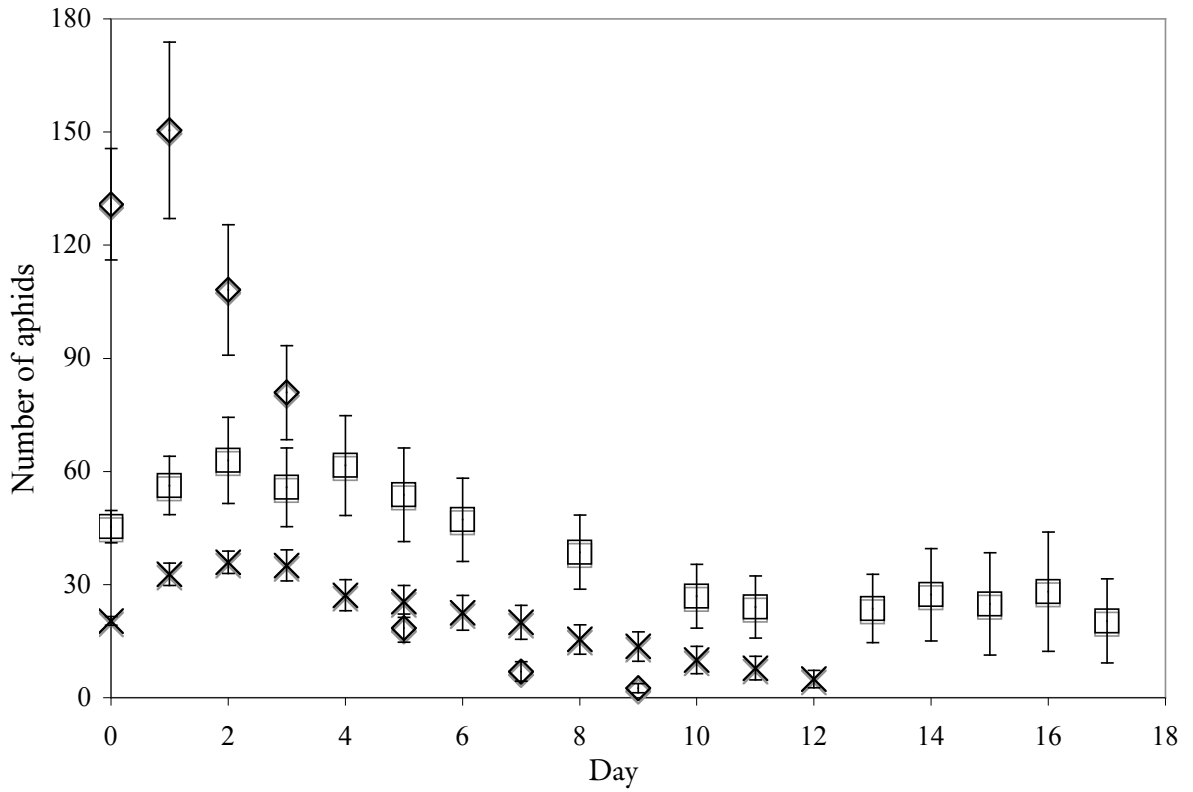


Figure 3. Mean (\pm SE) *T. aurantii* numbers per tip over time for 2005-2007 for ant-tended stems, \diamond 2005, \square 2006, \times 2007. Each line represents the combined average of *T. sessile* and *L. humile* tended aphid populations.

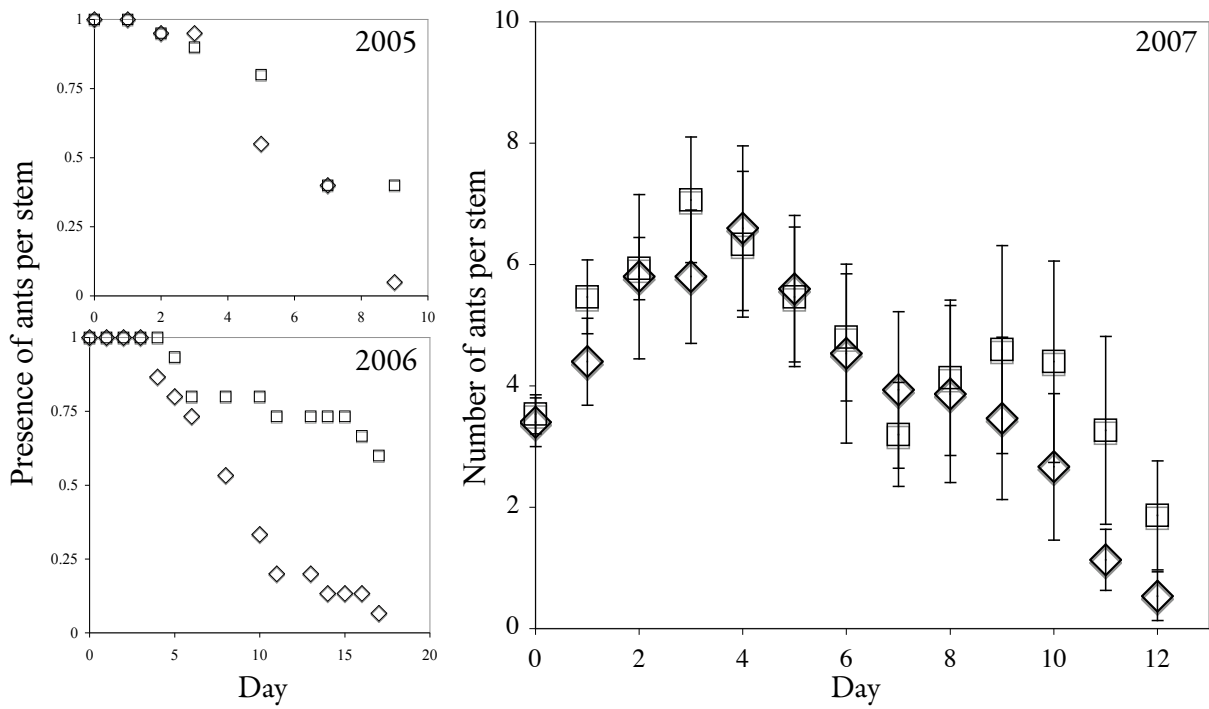


Figure 4. Ant presence and mean (\pm SE) ant numbers from 2005-2007. Year 2005 and 2006 shows ant presence per stem over time, \square *L. humile*, \diamond *T. sessile*. 1=at least one ant present, 0=no ants present, and each data point is the average presence across the treatment recorded at that point in time. Year 2007, represents the mean (\pm SE) number of ants per tip over time.

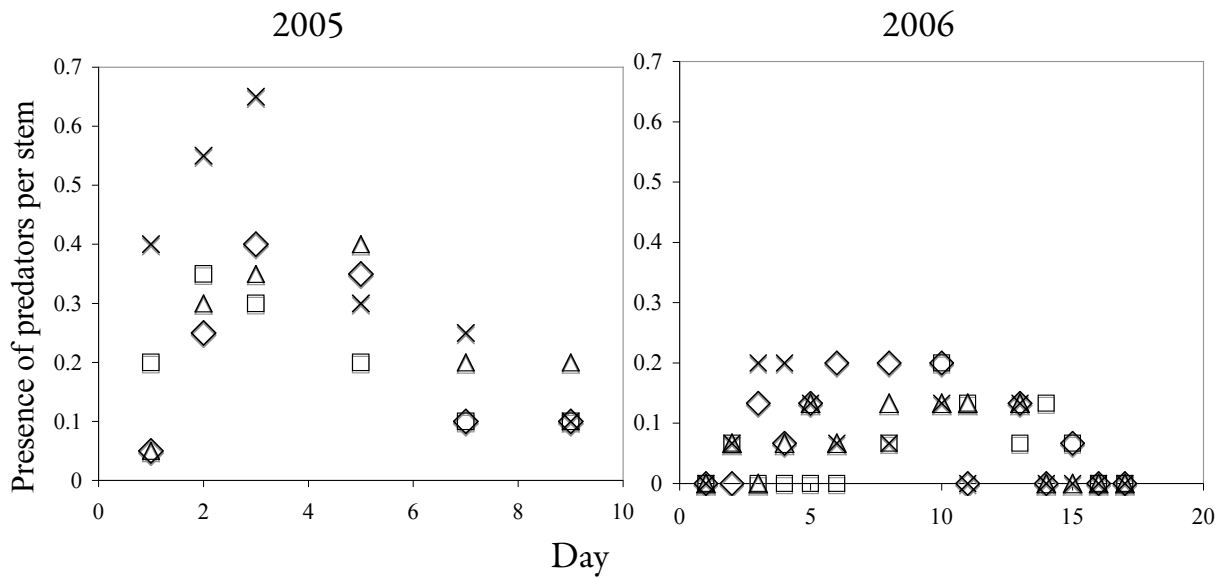


Figure 5. Average predator presence per stem for 2005 and 2006 over time, □ *L. humile* present, ◇ *T. sessile* present, × *L. humile* exclusion, △ *T. sessile* exclusion. 1=at least one predator present, 0=no ants present, and each data point is the average presence across the treatment recorded at that point in time.

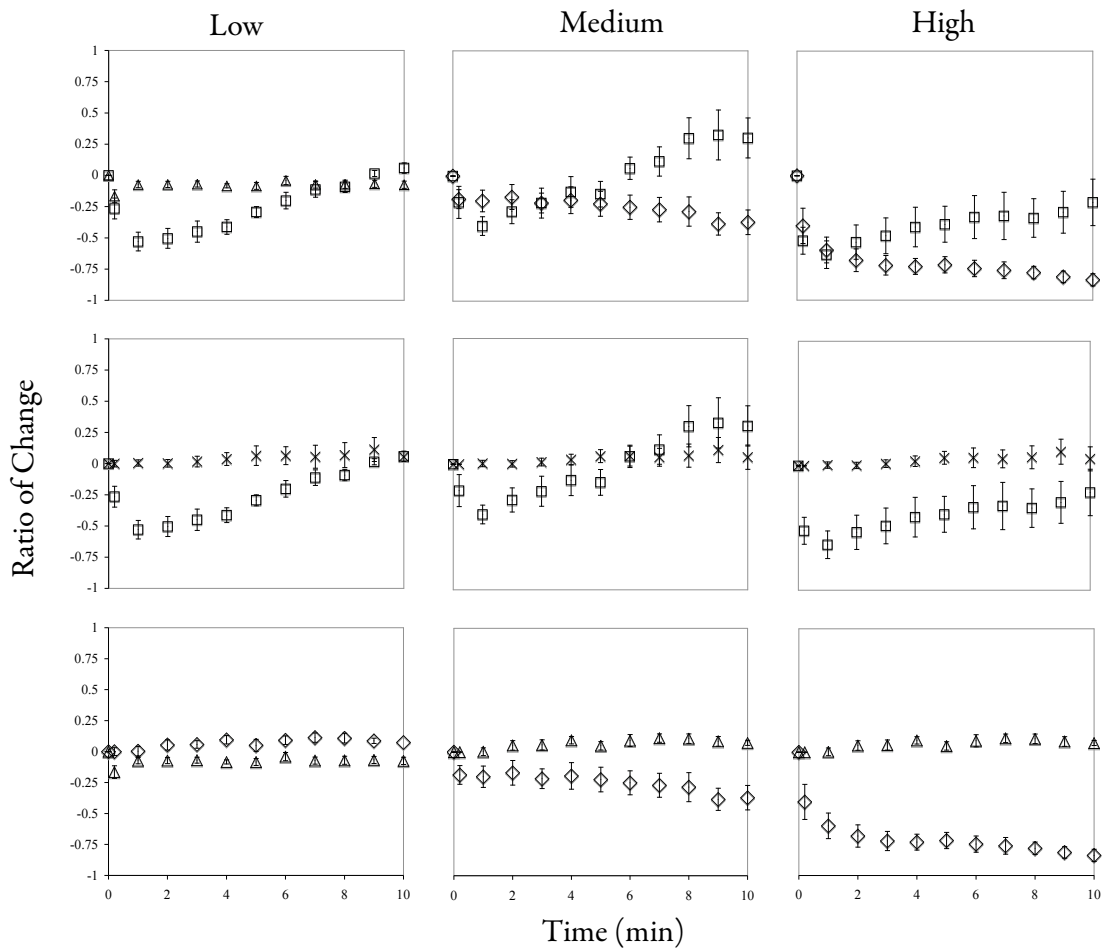


Figure 6. Mean (\pm SE) ratio of change of ant numbers in response to various flow rates of simulated rainfall disturbance, \square *L. humile* present, \diamond *T. sessile* present, \times *L. humile* no rainfall, \triangle *T. sessile* no rainfall. Columns represent different flow rates, left to right, low (0.175 ml/sec), medium (0.283 ml/sec) and high (0.55 ml/sec), respectively. The top row represents response of *T. sessile* and *L. humile*. The middle row represents ant response of *L. humile* and respective water-less controls. The bottom row represents ant response of *T. sessile* and respective water-less controls.

Table 1: Contrasts of aphid population growth rate between ant treatments and exclusion controls F-statistics, with numerator and denominator degrees of freedom subscripted, for contrasts between treatments and controls for aphid population growth rate for 2005-2007. *p<0.05, **p<0.001, ***p<0.0001, PROC MIXED repeated measures analysis.

Aphid population growth rate	2005	2006	2007
<i>L. humile</i> vs. <i>T. sessile</i>	F _{1,472} =1.27	F _{1,832} =11.55**	F _{1,712} =0.12
<i>L. humile</i> vs. <i>L. humile</i> exclusion	F _{1,472} =1.61	F _{1,832} =7.11*	F _{1,712} =15.72***
<i>T. sessile</i> vs. <i>T. sessile</i> exclusion	F _{1,472} =1.91	F _{1,832} =0.31	F _{1,712} =22.71***

Table 2: Contrasts of coccinellid predator activity between ant treatments and exclusion controls. Chi-square values for predator presence data in 2005 and 2006, for contrasts between treatments and controls. No significant p-values below the 0.05 alpha level were found, PROC GENMOD repeated measures analysis.

Predator abundance	2005	2006
<i>L. humile</i> vs. <i>T. sessile</i>	$\chi^2=2.72$	$\chi^2<0.01$
<i>L. humile</i> vs. <i>L. humile</i> exclusion	$\chi^2=0.95$	$\chi^2=2.04$
<i>T. sessile</i> vs. <i>T. sessile</i> exclusion	$\chi^2<0.01$	$\chi^2<0.01$

Table 3: Contrasts of ant treatments and water excluded controls during rainfall disturbance trials. F-statistics, with numerator and denominator degrees of freedom subscripted, for contrasts between treatments and controls for ant behavioral response to simulated rainfall disturbance. ‡p=0.07, *p<0.05, **p<0.001, ***p<0.0001, PROC MIXED repeated measures analysis.

Response to simulate rainfall	Low flow rate	Medium flow rate	High flow rate
<i>L. humile</i> vs. <i>T. sessile</i>	F _{1,784} =5.01*	F _{1,784} =31.90***	F _{1,784} =38.25***
<i>L. humile</i> vs. <i>L. humile</i> control	F _{1,784} =3.32‡	F _{1,784} =19.02***	F _{1,784} =7.19*
<i>T. sessile</i> vs. <i>T. sessile</i> control	F _{1,784} =0.03	F _{1,784} =3.98*	F _{1,784} =9.46*