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Examining Competitive Interactions Between Rasberry Crazy Ants (*Paratrechina* sp. nr. *pubens*) and Red Imported Fire Ants (*Solenopsis invicta*) Using Laboratory and Field Studies

by

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ABSTRACT

Examining Competitive Interactions Between Rasberry Crazy Ants (*Paratrechina* sp. nr. *pubens*) and Red Imported Fire Ants (*Solenopsis invicta*) Using Laboratory and Field Studies

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Studying nonnative species soon after their introduction is critical to understanding their risk of becoming widely invasive and determining effective methods of control. I conducted laboratory and field experiments on the Rasberry crazy ant (*Paratrechina* sp. nr. *pubens*), which was introduced to Pasadena, TX in 2002 and has since been spreading rapidly. These experiments focused on intraspecific aggression, as well as individual and colony-level interactions between crazy ants and red imported fire ants (*Solenopsis invicta*), which are dominant in the crazy ant's introduced range. Crazy ants displayed no intraspecific aggression. In individual aggressive encounters with fire ants, crazy ants had higher mortality than fire ants, but in colony-level clashes, crazy ants had less mortality and better control of food resources. These findings suggest that crazy ant abundance is key to their competitive success, and fire ants may provide biotic resistance to crazy ants in some areas.

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provided assistance in the field and much-needed distractions during the final few months of the thesis process. My father, John, also managed to call and cheer me up at just the right moments and to ask me science questions that reminded me why I started the thesis in the first place. My stepfather Jim always had supportive words. And when my mother Annette unknowingly let me raise my first ant colony in my bedroom on a stash of hidden Halloween candy, she probably could not have guessed that one day I would be studying ants full time. I appreciate her interest in my work and her warm and kind words that urged me on so many times when I doubted myself.

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Chapter 1

Introduction

Invasive species impact ecosystems and the economy. In the United States alone, control efforts directed at invasive species and environmental damage caused by invasive species total $137 billion annually (Pimentel et al. 2000). Ants are among the worst invaders and have caused ecological damage and economic costs worldwide (Lowe et al. 2004). Invasive ants often become highly abundant in their introduced ranges, outnumbering native ants and reducing native ant diversity (Holway et al. 2002), as well as affecting other invertebrates and vertebrates such as birds and lizards (Holway et al. 2002, Kenis et al. 2009). Preventing introductions is the most effective way to control the spread of invasive species, yet non-native ants continue to be accidentally introduced to new environments around the world. Therefore it is important to study the dynamics of new introductions in order to assess the risk of widespread invasion and to determine the most effective methods of control, as resources for controlling invasive species are often limited.

Despite considerable research on invasive species, one area that needs further exploration is how established invasive species influence the establishment and spread of newly introduced species. In some cases, existing invasive species have been shown to facilitate new invasions, which can lead to invasional meltdown (Simberloff and Von Holle 1999, O'Dowd et al. 2003, Bourgeois et al. 2005). However, in cases where established invasive species act as competitors or predators of newly introduced species, the established species may provide biotic resistance to new invasions (Simberloff and Von Holle 1999). Nevertheless, very few studies have looked at competition between invasive species in their shared introduced range (but see Belote and Weltzin 2006 and Griffen et al. 2008), and to my knowledge no studies have explicitly tested the negative effects of long established species on new invaders. Therefore, I tested for biotic resistance to a new introduction of a non-native species by an established invasive species, using ants as a study system.

In 2002, the Rasberry crazy ant, *Paratrechina* sp. nr. *pubens*, was unintentionally introduced to an industrial area along a ship channel in Pasadena, TX (Meyers 2008). Since its introduction to Texas, populations of Rasberry crazy ants have spread by 20-30m per month (Meyers 2008), and as of 2008 crazy ants had been reported in the following Texas counties: Bexar, Brazoria, Fort Bend, Chambers, Galveston, Hardin, Harris, Jefferson, Jim Hogg, Liberty, Montgomery, Orange, Walker and Wharton (Texas A&M 2008). The high rate of range expansion, along with the presence of extremely high densities of crazy ants in invaded areas, suggest that this exotic ant has the potential to become widespread and invasive. Additionally, very few pest control products, none of which are directly available to consumers, have been found to be effective in controlling crazy ant invasions (Texas A&M 2008).

Little is known about this species, including its native range and precise identification. Taxonomists have observed morphological and behavioral differences between Texas populations of *P.* sp. nr. *pubens* and the previously described *P. pubens*, yet the classification of *P.* sp. nr. *pubens* as *P. pubens* or an undescribed species remains inconclusive (Meyers 2008). The biology of the crazy ant is largely unknown, but the species is known to be polygynous and to nest ephemerally. Though crazy ant colonies are known to produce winged males and females, nuptial flights have never been observed in the native range (Texas A&M 2008).

Because so little is known about the ecology of this exotic ant, I conducted a series of laboratory and field experiments designed to explore the nature of the intraspecific interactions between Rasberry crazy ants, as well as the individual and colony-level competitive ability of crazy ants in comparison to the dominant ant species in its introduced range, the red imported fire ant, *Solenopsis invicta*, which is also an introduced species. Together these experiments addressed the issues of unicoloniality, the effect of diet on aggressive encounters, resource acquisition, and most importantly, biotic resistance by fire ants to the crazy ant invasion. In Chapter 2, I performed a series of aggression assays between crazy ants and fire ant workers of different sizes, between crazy ant workers from colonies receiving one of two protein sources and high or low doses of sugar, and between fire ants and crazy ant workers receiving the different diets. I found that large fire ants engaged in fewer fights than small fire ants, but fire ant size affected neither fire ant nor crazy ant mortality. On average, crazy ants experienced higher mortality than fire ants after antagonistic interactions. Diet did not induce aggressive interactions between crazy ants, but sugar level did play an important role in antagonistic interactions with fire ants, as crazy ants on a low sugar diet earned higher aggression scores.

The experiments in Chapter 3 examined colony-level interactions between crazy ants and fire ants both in the field and in the laboratory. In the laboratory experiment, colonies of fire ants and crazy ants were standardized by worker number or by biomass and used as either controls or in competing pairs that had access to a single set of protein and sugar baits. For both colony sizes, and in both control and competition settings, crazy ants recruited more quickly and in higher abundances to baits. Crazy ants also controlled significantly more baits than fire ants did at the end of the experiment. These results suggest that crazy ants, when evenly matched in colony size with fire ants, are capable of both discovering baits first and excluding fire ants from them, a combination of traits that has not been reported in native ants, but has been shown in invasive Argentine ants (Davidson 1998). To further test these findings, I conducted a field experiment where small crazy ant colonies were moved from a heavily invaded site to another location 750m away that had not yet been invaded. I then monitored the ant recruitment to sugar and protein baits for three weeks. Crazy ants discovered baits more quickly, but fire ants controlled the majority of baits and recruited to both sugar and protein in higher abundances than crazy ants. Crazy ant recruitment increased through time in crazy ant addition locations, but fire ant recruitment decreased in both control and crazy ant addition locations, suggesting that crazy ants are responding to, rather than causing, drops in fire ant recruitment.

In sum, the results of Chapters 2 and 3 suggest that crazy ant abundance is likely an important factor in competitive interactions with fire ants. On an individual level, fire ants are more successful fighters than crazy ants, but as colony units, crazy ants are more successful at resource retrieval and engage in more fights without experiencing correlated mortality when crazy ant and fire ant colonies are of similar size. The field experiment reinforced this hypothesis, as the small crazy ant colonies did not impact the recruitment to baits by fire ants from established nests. These findings suggest that fire ants may provide biotic resistance to crazy ants in areas that do not support high crazy ant densities, a factor which may severely restrict the potential spread of crazy ant populations. Putting my results in the context of observations from the field, I suggest that the future of the crazy ant invasion in southeast Texas will likely consist of coexistence with fire ants, likely in a patchy distribution of habitat partitioning caused by environmental factors that affect crazy ant abundance. Further studies, such as large-scale surveys of invaded sites and closer examinations of invasion fronts, would be useful in providing additional merit to this hypothesis. Nevertheless, these studies provide a crucial first exploration of the future of the crazy ant invasion, as well as a demonstration of the importance of competition between long-established invasive species and newly introduced species.

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Chapter 2

The Effect of Diet on Intra- and Interspecific Interactions of the Rasberry Crazy Ant (*Paratrechina* sp. nr. *pubens*)

Abstract

Biotic interactions are often important in the establishment and spread of invasive species. In particular, competition with species in the introduced range of an exotic species can be significant in preventing an exotic species from colonizing areas of suitable habitat (Walters and Mackay 2005, Menke et al. 2007). Here we examined interference competition in the Rasberry crazy ant, *Paratrechina* sp. nr. *pubens*, which was recently introduced to the Gulf Coast of Texas, by studying intraspecific interactions between nests of Rasberry crazy ants and interspecific interactions with the red imported fire ant, *Solenopsis invicta*. Specifically, we examined individual-level interactions in three sets of ants: 1) between crazy ant workers and either small (minor) or large (major) fire ant workers, 2) between crazy ant workers from different nests receiving one of two protein sources and either high or low doses of sugar, and 3) between fire ants and crazy ant workers receiving the different diets. We found that large fire ants fought less often with crazy ants than did small fire ants, but fire ant size affected neither fire ant nor crazy ant mortality. Overall, crazy ants experienced higher mortality than fire ants after antagonistic encounters. Differences in diet did not alter interactions between crazy ant workers from different nests, but sugar level did play an important role in antagonistic interactions with fire ants, as crazy ants on a low sugar diet earned higher aggression scores. Our findings suggest that fire ants may outcompete crazy ants on an individual level, providing some biotic resistance to crazy ant range expansion. However, fire ants

may overcome by individual crazy ants that have a restricted sugar intake, which may occur when crazy ants are excluded from resources by fire ants.

Introduction

Biotic interactions between introduced species and native or pre-established exotic species are important influences on the success and spread of invasive species (Mack et al. 2000, Mitchell and Power 2003, Ness and Bronstein 2004, Torchin and Mitchell 2004). Competition from established species in the introduced range, like between ant species for food or territory, can sometimes serve as biotic resistance to invasive species (Walters and Mackay 2005, Menke et al. 2007). Competitive interactions can be exploitative, where individuals compete indirectly via their effects on shared resources, or interference, where individuals directly clash through antagonistic behaviors (Ricklefs 2001). In ants, interference competition is common and can cause death or injury of workers and loss of access to food or territory (Savolainen and Vepsalainen 1988, Hölldobler and Wilson 1990, Andersen and Patel 1994). Therefore, interference competition can be an important factor in the spread and success of an exotic ant pest, such as the recently introduced Rasberry crazy ant, *Paratrechina* sp. nr. *pubens*. In this paper, I examine direct intraspecific competitive interactions between crazy ant nests and direct interspecific competitive interactions between crazy ants and an extremely common invasive ant in its introduced range, *Solenopsis invicta*, the red imported fire ant. Intraspecific and interspecific interactions of Rasberry crazy ants have never been examined, yet the ant is a pest species that is increasing its introduced range southeast of Houston, TX. To my knowledge, no studies have examined competition between long established invasive species and newly introduced species for evidence of biotic resistance, yet the invasive fire ants may provide biotic resistance to the spread of Rasberry crazy ants. Therefore it is important to investigate antagonistic encounters both between Rasberry crazy ants nests and with fire ant workers.

Because nestmate recognition, territoriality, and activity level are influenced by diet (Davidson 1997, Howard and Blomquist 2005, Buczkowski and Silverman 2006, Grover et al. 2007), the diet of an ant can influence aggressive interactions, whether between species or colonies of a single species. The cuticular hydrocarbons of prey items can be transferred to ants that come into contact with it, disrupting the ant's hydrocarbon profile and affecting nestmate recognition (Buczkowski et al. 2005). Differences in prey type have been shown to induce aggression between nestmates (Silverman and Liang 2001, Corin et al. 2007, Sorvari et al. 2008) and to affect antagonistic interactions between nests or colonies of a given species (Liang et al. 2001, Buczkowski and Silverman 2006, Grover et al. 2007), though the effect of diet on interspecific antagonistic interactions has not been extensively studied (but see Alloway et al. 1991), and the effect of diet on competitive interactions between two invasive ant species has never been studied. Additionally, it has been proposed that invasive ants consume higher amounts of carbohydrate resources than native ants, which may increase the tempo of their worker activity, contributing to their high levels of abundance and domination of resources and territory (Holway et al. 2002). The amount of sugar in a colony's diet has been shown to be an important variable in activity level and antagonistic behavior in both *Formica aquilonia* and invasive Argentine ants, *Linepithema humile* (Grover et al. 2007, Sorvari et al. 2008). Because of the importance of diet in interactions between ants, we examined the effects of both sugar level and protein type on intra- and interspecific competition in Rasberry crazy ants.

Paratrechina sp. nr. *pubens* (Hymenoptera, Formicidae, Formicinae) was first discovered in an industrial area along the ship channel in Pasadena, TX in 2002 (Meyers 2008). Little is known about this ant species, including its exact species identification and its native range. Ant taxonomists have noted differences between Texas populations of Rasberry crazy ants and the previously described *P. pubens*, yet morphological and behavioral differences between these ants have proven to be inconclusive in determining if *P.* sp. nr. *pubens* is *P. pubens* or an undescribed species (Meyers 2008). Since its introduction to Texas, the range of Rasberry crazy ants has increased by 20-30m per month (Meyers 2008). Media reports on the ant (named after the first pest control agent to discover the invasion, Tom Rasberry) focus on the tendency of Rasberry crazy ants to nest in electronics and cause short circuits. Though these effects of the crazy ants are likely overstated, it is known that Rasberry crazy ants are often found in extremely high densities in invaded areas (Meyers 2008). Like another well-known invader, the Argentine ant (Holway et al. 1998), crazy ant populations appear to be unicolonial; colony boundaries seem to be nonexistent as ants move freely between nests (Hölldobler and Wilson 1990). Crazy ants are often found in areas that would be suitable habitat for red imported fire ants (personal observation, K. Horn), such as woods and open areas, suggesting that the two species may often come into contact and compete for resources.

Red imported fire ants (Hymenoptera, Formicidae, Myrmicinae) are themselves one of the world's top 100 worst invasive species (Lowe et al. 2004). Introduced to the United States via the port city of Mobile, AL in the 1930's (Buren et al. 1974), fire ants have since spread throughout more than 106 million hectares of the southeastern US, the Midwest, and California, where they are the dominant ant species in disturbed habitats (Williams 1994). Due to their harmful effects on humans, agriculture, and ecosystems, fire ants cost nearly $1 billion per year in economic losses and control efforts (Pimentel et al. 2000). Some news reports have suggested that crazy ants attack, eat, and displace fire ants, yet none of these claims have been tested. Due to the widespread invasion of fire ants and the locally abundant populations of Rasberry crazy ants, interactions between these two species may be very important in affecting the spread of crazy ant populations.

To examine the intra- and interspecific interactions of the Rasberry crazy ants, the following sets of ant pairings were observed: 1) crazy ants and either small (minor) or large (major) fire ants workers, 2) crazy ants from nests in the same supercolony which had been isolated and fed one of two protein sources and either high or low doses of sugar water, and 3) crazy ants which had been on the different diets described above and fire ants. Two sizes of fire ants were used because both minor and major fire ants forage outside of the nest where they can interact with crazy ant foragers, and the different sizes of fire ants could have different fighting tactics or abilities which may affect their likelihood to engage in fights and/or their ability to kill ant opponents. All interactions were examined using aggression assays with 5 ants from each species or treatment in a Petri dish, which have been shown to be highly consistent and correlate well with full colony introductions (Roulston et al. 2003). Together, these three sets of aggression assays made it possible to address the following questions: 1) Are crazy ants or fire ants more successful in fights? 2) Is fighting success affected by fire ant size? 3) Do differences in diet affect interactions between workers of different crazy ant colonies? and 4) Can the diet of crazy ants affect antagonistic interactions with fire ants? Question three examines whether crazy ant unicoloniality can be affected by diet, while questions one, two, and four focus on whether fire ants may provide biotic resistance to further crazy ant colonization and spread. Together, the answers to these questions could have important implications for the future of the crazy ant introduction.

Methods

All colonies of Rasberry crazy ants used in aggression assays were collected in Pearland, TX (29.55°N, 95.28°W) on May 24 and May 31, 2008. Though 24 distinct nests were collected, because crazy ants at this site display no aggression between nests, it is likely that all nests are part of a large supercolony (Hölldobler and Wilson 1990). Fire ant colonies were collected from Katy, TX (29.93°N, 95.94°W) in early May 2008. Colonies of both species were polygynous. In order to separate ants from the nesting material with which they were collected, we flooded colonies and then transferred all ants to 24cm x 11cm plastic nest boxes. Nest boxes were ringed with a thin layer of Tanglefoot (Tanglefoot, Grand Rapids, Michigan, USA) near the top of the inside walls in order to prevent escapes.

The initial diet administered to both crazy ant and fire ant colonies consisted of freezer-killed mealworms and sugar water. This diet was maintained until July 18, 2008, when all food was removed from the crazy ant nest boxes and diet manipulations began. Each of the 24 crazy ant colonies was assigned to one of four treatments: cricket/high sugar, cricket/low sugar, wax worm/high sugar, or wax worm/low sugar, such that there were six colonies in each treatment. The low concentration sugar water consisted of 5 ml of sugar in 120 ml of water (4.2% sugar by volume); the high concentration sugar water consisted of 20 ml of sugar in 120 ml of water (16.7% sugar by volume). Each colony was given half of a freezer-killed cricket (Orthoptera, Gryllidae) or half of a freezer-killed wax worm and 7.4 ml of sugar water of the appropriate concentration every other day. Wax worms (Lepidoptera, Pyralidae) were supplied by Armstrong's Cricket Farm (West Monroe, LA) and crickets were purchased at a pet supply store. These two bait items have different nutritional value; wax worms are less than 23% protein by mass, while crickets are more than 50% protein by mass (Finke 2002, Wang et al. 2004). While crazy ants were receiving experimental diets, fire ant colonies were starved.

The following aggression assays were performed: 5 crazy ant workers in a Petri dish with either 5 minor or 5 major fire ant workers, 5 crazy ant workers from one diet treatment in a Petri dish with 5 crazy ants from a colony receiving a different diet treatment, and 5 minor fire ants with 5 crazy ants on an experimental diet. For each aggression assay, ants were placed in a 9-cm Petri dish with Fluon (polytetrafluoro-ethylene)-coated sides that prevented ant escape during these periods of observation. The Suarez scale (Suarez et al. 1999) was then used to score the behavior of pairs of interacting ants every minute for either 5 or 10 minutes, depending on the species combination: 0 –no interaction (i.e., ants ignored each other), 1 - touching with antennae, 2 - avoidance, 3 – aggression (such as biting antennae or legs), and 4 – fighting (both ants engaged). The average aggression score for each pairing was then calculated using all scores from the duration of observation, including the zeroes representing ants that were not interacting. The number of ants from each treatment or colony engaged in fights was also recorded each minute. In all combinations, the first observation was made five seconds after all ants were released into the Petri dish. At the end of the 5- or 10-minute observation period, the ants were then left in the Petri dish for 1 or 2 hours, and a mortality score was recorded after each hour. All statistical analyses were performed using JMP 7.0.2 (SAS Institute Inc., 2007). Each of the aggression assays is explained in further detail below.

Crazy ants vs. small and large fire ants

Aggression assays between crazy ant workers and fire ant workers of different sizes were conducted on July 16, 2008. Each of 12 fire ant colonies was used twice as a source of workers, the first time with 5 small workers paired up with 5 crazy ant workers, and the second time with 5 large workers paired up 5 crazy ant workers from a different colony, creating a total of 24 aggression trails. Aggression and fighting data were recorded for 10 minutes. Mortality was recorded after 10 minutes, and for 10 pairings, also after 1 hour.

In order to quantify the size difference between crazy ants and minor and major fire ant workers, the head length (from the front of the clypeus to the posterior margin of the head) of 10 ants from each of the 3 groups was measured. Head length is considered the most reliable predictor of body size across ant species (Kaspari and Weiser 1999). The average and standard error of the length was calculated for each group, and the head lengths were tested for a significant effect of ant type using a one-way ANOVA, followed by a post hoc Tukey's test to discern between the 3 types. The head lengths of each type of ant (crazy ant, small fire, and large fire ant) were all significantly different from one another ($F_{2,29} = 262.87$, $p = <0.0001$, Table 2.1). In order to estimate the size distribution of fire ant workers, between 21 and 36 fire ant workers from each of 10 colonies were classified as small, medium, or large. These classifications were based on visual estimates based on the range of worker sizes, rather than specific measurement ranges. The average percentage and standard deviation of workers in each category was calculated (Table 2.1).

Overall average aggression score, average number of fire ants fighting, and average number of crazy ants fighting all were analyzed using a one-way ANOVA to determine the effect of fire ant size on the response variables. Average mortality after 10 minutes was minimal and often zero, thus it was not analyzed. Mortality for both crazy ants and fire ants after 1 hour was non-normal and could not be transformed to achieve normality and therefore was analyzed for an effect of fire ant size using a Wilcoxon signed rank test. To determine if the average number of ants fighting differed by species, the difference between species in number of ants fighting was taken for each aggression assay and the resulting values were tested for significant deviation from zero using a Wilcoxon signed rank test. To test for a species difference in mortality after 1 hour, the differences were tested for deviation from zero using a 1-group, 2-tailed t-test. The differences for both response variables were also tested for a fire ant size effect using Wilcoxon signed rank tests. Additionally, average crazy ant and fire ant mortality after 1 hour was compared to survival of control Petri dishes containing either 5 crazy ants or 5 fire ants using Wilcoxon signed rank tests on the fire ant mortality and the square root of crazy ant mortality after 1 hour.

Crazy ants receiving different diets

Intraspecific aggression trials were conducted on July 29, 2008. A total of 24 pairings of colonies were used. Some source colonies were used more than once, but all pairings were unique. There were 24 total pairings: 8 replicates of pairings with the same sugar level but different protein type, 6 replicates of pairings with different sugar levels but the same protein type, 6 replicates of pairings differing in sugar level and protein type, and 4 replicates of pairings of the same sugar level and protein type. In order to distinguish between the two treatments of crazy ants in aggression trials, workers were coated with either pink or green fluorescent powder (Day Glo Color Corp. Cleveland, Ohio, USA) using a small paintbrush in an intermediate holding container a few minutes before they were added to the Petri dish. After the ants had ceased grooming and resumed moving about the container, they were placed into the Petri dish. Aggression and fighting data were recorded for 5 minutes, and the number of dead ants of each color was recorded at 5 minutes, 1 hour, and 2 hours. As a control, the average mortality of un-powdered crazy ants kept in a Petri dish for 2 hours was tested.

Because data were non-normal, even with transformations, a Wilcoxon signed rank test was used to determine if peak interaction score varied significantly when colony pairs differed by protein type, sugar level, both, or neither. The same test was also used to determine if diet differences affected mortality after 1 or 2 hours. Additionally, a Wilcoxon signed rank test was used to compare 1 and 2 hour mortality of all pairings of different colonies and powdered controls of 10 workers from the same colony and unmanipulated controls of 5 workers from the same colony.

Crazy ants receiving different diets vs. fire ants

In order to test the effect of crazy ant diet on interactions with fire ants, aggression assays were again performed on July 31, 2008, using 5 crazy ant workers and 5 small fire ant workers. Each crazy ant colony was used only once. Two fire ant colonies were used twice, but each colony pair was unique. There were 6 aggression assays performed for each of the 4 treatments for a total of 24 colony pairings. Aggression and fighting data were recorded for 5 minutes, and mortality was recorded at 5 minutes, 1 hour, and 2 hours.

To determine the effect of diet on aggression score, fighting, and mortality of the two species, 2-way ANOVA's were performed with protein type, sugar level, and the interaction of protein type and sugar level as factors. The following response variables had a normal distribution without transformation: the average interaction score across all time periods, the average number of crazy ants in engaged in fights, the average number of fire ants involved in fights, and crazy ant mortality after 2 hours. The mortality of fire ants after 2 hours was square root transformed prior to testing for a diet effect with an ANOVA. Mortality after 2 hours was used because it had higher values and a more normal distribution than 1-hour mortality, and in only 2 cases were all 5 of the ants from a colony in a Petri dish dead (both occasions were fire ants). In order to test for a difference between crazy ants and fire ants in average number of workers fighting and mortality after 2 hours, the fire ant values were subtracted from the crazy ant values for each response variable. A Wilcoxon signed rank test was used to test if the mean of the difference in numbers of workers fighting was significantly different from zero, and an ANOVA was used to determine if the mean of the difference in mortality after 2 hours was affected by sugar level, protein type, or an interaction of the two variables. Also, a

Wilcoxon signed rank test was used to see if protein or sugar level significantly affected the difference between the average number of crazy ant and fire ant workers engaged in fights.

Results

Crazy ants vs. minor and major fire ants

There was no effect of fire ant worker size category on overall average aggression score ($F_{1,22} = 1.20$, $p = 0.285$). The average aggression score of containers with large fire ants was 1.00 ± 0.11, and the average aggression score of dishes with small fire ants was 1.18 ± 0.11. Though these scores were low due to the number of ants ignoring the other species (receiving a score of 0), nearly all of the non-zero interactions were scored with 3's or 4's, indicating a high level of aggression during encounters. Though there was no effect of fire ant size category on average number of crazy ants fighting ($F_{1,22} = 2.91$, $p = 0.102$), there was a significant effect of fire ant size on the average number of fire ants fighting ($F_{1,22} = 4.76$, $p = 0.040$), with small fire ants having an average of 51.6% more workers fighting than large fire ants fighting during a given observation period (Figure 2.1). Fire ant size category had no effect on either crazy ant mortality ($Z_1 = 0.95$, $p = 0.329$) or fire ant mortality ($Z_1 = 1.72$, $p = 0.190$) after 1 hour.

Comparing the response variables of the two species, there was a trend for crazy ants to have a higher average number of workers engaged in fights ($Z_{23} = 12.00$, $p = 0.063$, Figure 2.2), and crazy ants had higher mortality than fire ants after 1 hour ($t_9 = 2.4140$, $p = 0.039$, Figure 2.3). Fire ant size did not have an effect on the difference between crazy ants and fire ants for average number of ants fighting ($Z_1 = -1.23$, $p =$

0.208) or mortality after 1 hour ($Z_1 = -0.43$, $p = 0.670$). Crazy ant mortality when fighting fire ants was significantly higher than that of control crazy ants ($Z_1 = -2.65$, $p = 0.008$). Controls averaged 0.2 ± 0.2 dead crazy ants after 1 hour, whereas crazy ants fighting fire ants had an average mortality of 2.7 ± 0.45 ants after 1 hour. Likewise, fighting fire ants had significantly higher mortality after 1 hour than controls ($Z_1 = -2.01$, $p = 0.037$).

Crazy ants receiving different diets

There was no fighting between crazy ants in any of the aggression assays; the highest aggression score was a 2, which occurred only 5 times during the 144 observation periods. Peak interaction score therefore was not significantly affected by diet treatments ($Z_3 = 1.57$, $p = 0.667$). There was no mortality for any of the ants after 5 minutes. Neither mortality after one hour ($Z_3 = 2.95$, $p = 0.340$) nor mortality after two hours ($Z_3 = 2.42$, $p = 0.491$) were significantly affected by diet treatments. The overall average mortality after two hours was 1.09 ± 0.21 out of 5 ants. This was not significantly different from the average two-hour mortality of the five sets of unpowdered ants in a Petri dish ($Z_1 = 0.15$, $p = 0.694$). Additionally, the 4 pairs that received the same diet did not have significantly different mortality than the 5 unpowdered controls ($Z_1 = 0.00$, $p = 1.000$).

Crazy ants receiving different diets vs. fire ants

Sugar level significantly affected the average interaction score ($F_{1,20} = 4.97$, $p = 0.037$), though protein type had no effect ($F_{1,20} = 0.75$, $p = 0.398$) and the interaction of the two factors was not significant ($F_{1,20} = 1.16$, $p = 0.294$). Ants receiving a lower sugar diet had higher aggression scores when interacting with fire ants than did high sugar crazy ants (Figure 2.4). There was no effect of sugar level on the on the number of crazy ants ($F_{1,20} = 3.16$, $p = 0.091$) or fire ants ($F_{1,20} = 3.28$, $p = 0.085$) engaged in fights. Protein type and the interaction of the two resources were not significant factors in either the number of crazy ants fighting ($F_{1,20} = 0.10$, $p = 0.757$ for protein; $F_{1,20} = 1.27$, $p = 0.2730$ for the interaction) or the number of fire ants fighting ($F_{1,20} = 0.22$ and $p = 0.644$ for protein, $F_{1,20} = 0.95$ and $p = 0.341$ for the interaction). After 2 hours, there was no effect of sugar level on crazy ant mortality ($F_{1,20} = 3.19$, $p = 0.090$). Neither protein type ($F_{1,20} = 0.09$, $p = 0.769$) nor the interaction of sugar and protein ($F_{1,20} = 0.09$, $p = 0.769$) significantly affected crazy ant mortality, and none of the response variables affected fire ant mortality ($F_{1,20} = 0.21$, $p = 0.651$ for sugar level; $F_{1,20} = 2.84$, $p = 0.108$ for protein type; $F_{1,20} = 0.59$, $p = 0.453$ for the interaction).

Overall, there was no difference between species in average number of workers fighting ($Z_{23} = 9.00$, $p = 0.289$) or mortality ($t_{23} = -1.14$, $p = 0.268$). There was no effect of sugar level ($F_{1,20} = 2.49$, $p = 0.130$), protein type ($F_{1,20} = 0.90$, $p = 0.354$), or the interaction of diet components ($F_{1,20} = 0.54$, $p = 0.470$) on the difference between crazy ants and fire ants in mortality. There was also no effect of protein or sugar level (both $Z_1 = -0.73$, $p = 0.444$) on the difference in average number of fighting ants of each species.

Discussion

The results of the trials between crazy ants and fire ants of different sizes showed a trend for more crazy ants than fire ants to be involved in fights (Figure 2.2) and higher crazy ant than fire ant mortality after one hour (Figure 2.3). Both ant species experienced higher mortality after exposure to the other species than either species experienced in isolation. Fire ant worker size had little effect on antagonistic interactions with crazy ants, as aggression score, the number of crazy ants fighting, and mortality for both species were not influenced by fire ant size. The exception is that small fire ants fought more than 50% more often than large fire ants (Figure 2.1). The tendency of small fire ant workers to fight more often may be biologically important, as small fire ants are nearly 3 times more common than large fire ants based on the average distribution of worker sizes in our field-collected colonies (Table 2.1). This may mean that crazy ants are more likely to encounter small workers that readily engage in fights than large workers that avoid fights, especially since smaller fire ant workers have been found ot forage farther from nests (Martin and Vinson 2008). The increase in fire ant fighting does not correlate with an increase in mortality for either crazy ants or fire ants, suggesting that the likelihood of engaging in fights may not be indicative of the outcome of interactions. Overall, crazy ants, which spray formic acid with an acidopore, had an average mortality nearly twice that of fire ants (Figure 2.3), which inject acid using a stinger (Hölldobler and Wilson 1990). This large average difference was not statistically significant perhaps due to the small sample size and the low power of a non-parametric test, and it is possible that the difference in mortality between the two species is biologically significant. Though spraying acid can be a very effective method of attacking other insects, as the acid is absorbed through the exoskeleton, crazy ants may have less success in fights with fire ants due to the difference in size, as even small fire ant workers are significantly larger than crazy ants. If the advantage of fire ants over crazy ants in individual clashes is biologically significant, it could be important in limiting the establishment and spread of crazy ants in habitats where fire ants are common.

Diet differences had no effect on intraspecific crazy ant interactions after 12 days. No fighting was observed between any of the workers, and the highest scoring interaction recorded was avoidance, which occurred only 5 times. Mortality did not differ among pairs that received different diets, pairs receiving the same diet, and controls of 5 ants. The lack of antagonistic interactions between crazy ants that received different diets may indicate that diet changes alone do not disrupt the chemical profile of the workers enough to overcome the unicoloniality of the introduced population. However, several studies that have examined the effect of diet on intraspecific interactions have found that aggression can be induced between unicolonial nests or former nestmates (Liang et al. 2001, Silverman and Liang 2001, Buczkowski and Silverman 2006, Corin et al. 2007). For example, Corin et al. (2007) found aggression between unicolonial Argentine ant nests after 56 days of diet maintenance, and Silverman and Liang (2001), who studied interactions between separated nestmates consuming different prey at several intervals for 56 days, found that former nestmates fought each other after 28 days. Additionally, Lim et al. examined interactions between nestmates of *Paratrechina longicornis* and found antagonistic behavior beginning at 21 days after the implementation of diet treatments (Lim et al. 2003). Together, these studies could suggest that the absence of intraspecific aggression in Rasberry crazy ants is due to the treatments not being maintained long enough to for nestmate recognition to be affected. Nevertheless, one study of Argentine ants showed antagonistic behavior between nestmates after as little as 2 minutes of contact with a prey item, the brown-banded cockroach, *Supella longipalpa* (Liang et al.

2001). Therefore, changes in nestmate recognition are therefore conceivable within 12 days, and the protein sources used in the diets, though from different orders of insects, may not have had distinct enough chemical profiles to affect nestmate recognition. Alternatively, crazy ant nestmate recognition may be based primarily on genetic cues which may not be significantly affected by environmental factors like diet, as is the case with Argentine ants (Suarez et al. 2008 and references therein). Ultimately, further study using longer periods of diet maintenance and different protein types, such as cockroaches, may affect nestmate recognition in Rasberry crazy ants, though this study did not reveal any evidence of inducible intraspecific aggression. As the density of colonies increases, this lack of intraspecific aggression may allow continued population growth.

In keeping with the results of the intraspecific aggression assays, diet had only a limited effect on interactions between crazy ants and fire ants. Crazy ants receiving a low sugar diet engaged in higher scoring aggressive interactions with fire ants than crazy ants on a high sugar diet (Figure 2.4). Yet sugar level did not affect the number of ants fighting or mortality for either species. Protein type had no effect on antagonistic interactions, which is consistent with the findings of Alloway et al. (1991), who found no difference in the number of fighting ants of two species of Leptothoracine ants that were fed diets that differed in protein type and vitamin and mineral source but not sugar source. Overall, the two species did not differ significantly in the average number of workers fighting or in mortality after one hour, and there was no effect of diet on the difference between the two species.

Carbohydrates play an important role in ant activity levels (Grover et al. 2007). Therefore, it is expected that sugar level would affect interspecific antagonistic interactions. What may seem surprising is that the data show that crazy ants receiving fewer carbohydrates engage in more aggressive interactions with fire ants (Figures 2.3). However, in a study examining the effect of diet on intraspecific interactions in Argentine ants, Grover et al. (2007) found that aggression was decreased in the absence of sugar, but there was no difference in aggression scores between low sugar and high sugar treatments. Furthermore, per capita activity (based on the exploration of a structure not containing food) was higher in the low sucrose treatment than in either the sucrose-free treatment or the high sucrose treatment. In light of these findings, it is conceivable that crazy ants that are sugar-limited may experience increased per capita activity and therefore engage in more intense fights with another species, in this case fire ants. The fact that there was no effect of sugar level on mortality suggests that fighting intensity is not closely correlated with mortality for Rasberry crazy ants. Overall, the finding of increased aggression score on a low sugar diet provides new insight in the behavior of crazy ants, showing a similarity to the highly invasive Argentine ant (Grover et al. 2007).

The findings of this study give insights on the biotic factors affecting the spread of the introduced Rasberry crazy ant populations. The most common ant in the introduced range, the red imported fire ant, has less mortality when facing off in fights with crazy ants. Additionally, small fire ants, which are most common, engage in fights more often than larger fire ants. Together, these results suggest that fire ants may have a competitive advantage over crazy ants and may be able to defend their territory from a neighboring colony of crazy ants, though colony-level studies will put the individual-level advantage in the context of relative abundance and worker activity. On the other hand, when crazy ants consume a reduced sugar diet, they fight more intensely, without a corresponding increase in mortality. Therefore, a crazy ant colony that has been competitively excluded from sugar sources by fire ants or other ants may engage intense fights, inflicting various degrees of mortality on other ant species without suffering high mortality themselves. Combined with the fact that differences in diet showed no sign of affecting nestmate recognition, large supercolonies of sugar-limited crazy ants may challenge fire ant dominance in certain areas by overwhelming fire ant nests with considerable numbers of very active workers that readily engage in fights. Nevertheless, because fire ants have almost half as much mortality as crazy ants in individual interactions, fire ant colonies could provide biotic resistance to the crazy ant invasion in places where fire ants outnumber crazy ants and can afford to engage in fights without serious losses in workers or foraging efficiency. In sum, these findings help explain the occurrence of large supercolonies of crazy ants but suggest that established fire ants may provide some biotic resistance to crazy ants, especially if crazy ants are not sugar-limited. Further studies on colony-level interactions will further elucidate the ability of an established invasive species to resist the spread of a newly introduced species.

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Table 2.1. Head lengths of fire ants and crazy ants and fire ant colony size distribution. Ants that do not share the same level letter significantly differ in head length (as indicated in a Tukey's post hoc test). Average # is the number of fire ants in each size class in a haphazardly selected sample of 21 to 36 ants from 10 colonies. Percentages are from an average sample size of 28.36 ants. Errors are ± 1 SE.

Ant	Length (mm)	Level	Average #	% of Sample
Large fire	1.28 ± 0.02	A	4.73 ± 5.02	16.1%
Medium fire	N/A	N/A	10.91 ± 7.46	37.2%
Small fire	0.78 ± 0.02	B	12.72 ± 5.48	46.7%
Crazy	0.69 ± 0.01	C	N/A	N/A

Figure 2.1. The effect of fire ant worker size on the average number of fire ants fighting during each observation (out of a total of 5 fire ants). Small fire ants fought more often than large fire ants (p = 0.040). Error bars indicate + 1 SE.

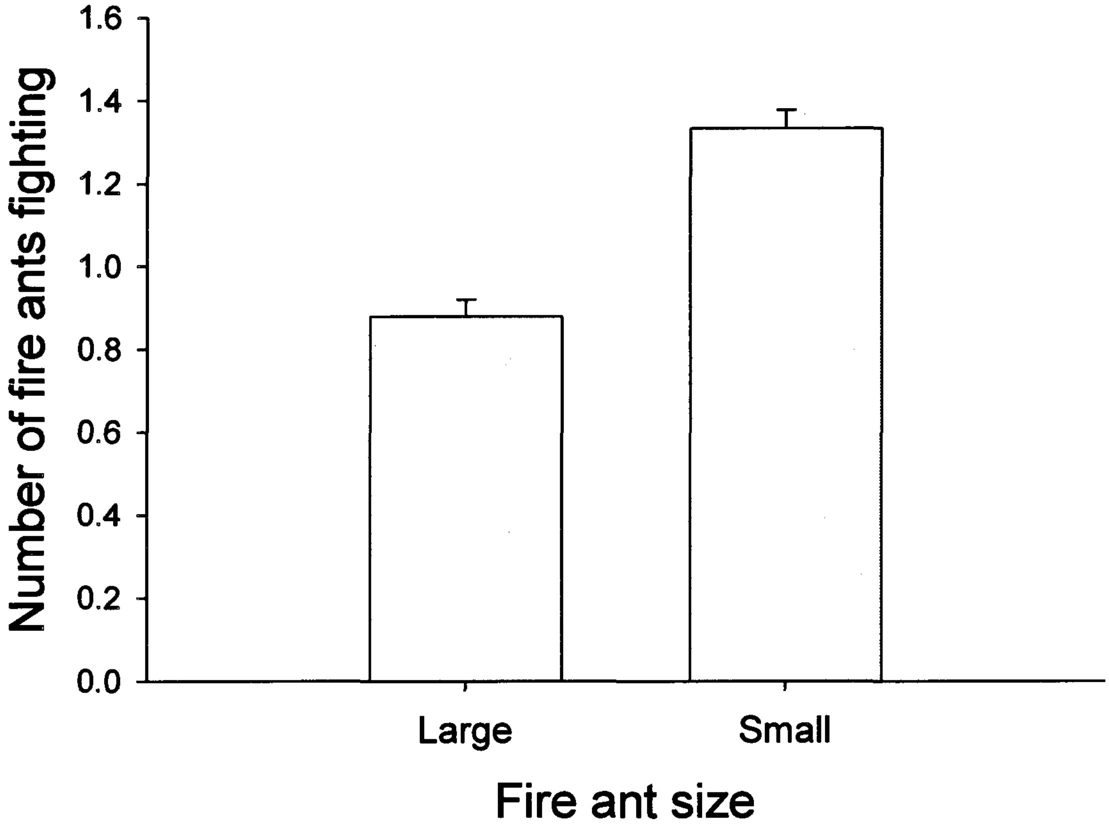


Figure 2.2. The number of Rasberry crazy ants and fire ants engaged in fights. On average, crazy ants had more workers engaged in fights than fire ants did in aggression trials between the two species (p = 0.063). Error bars indicate + 1 SE.

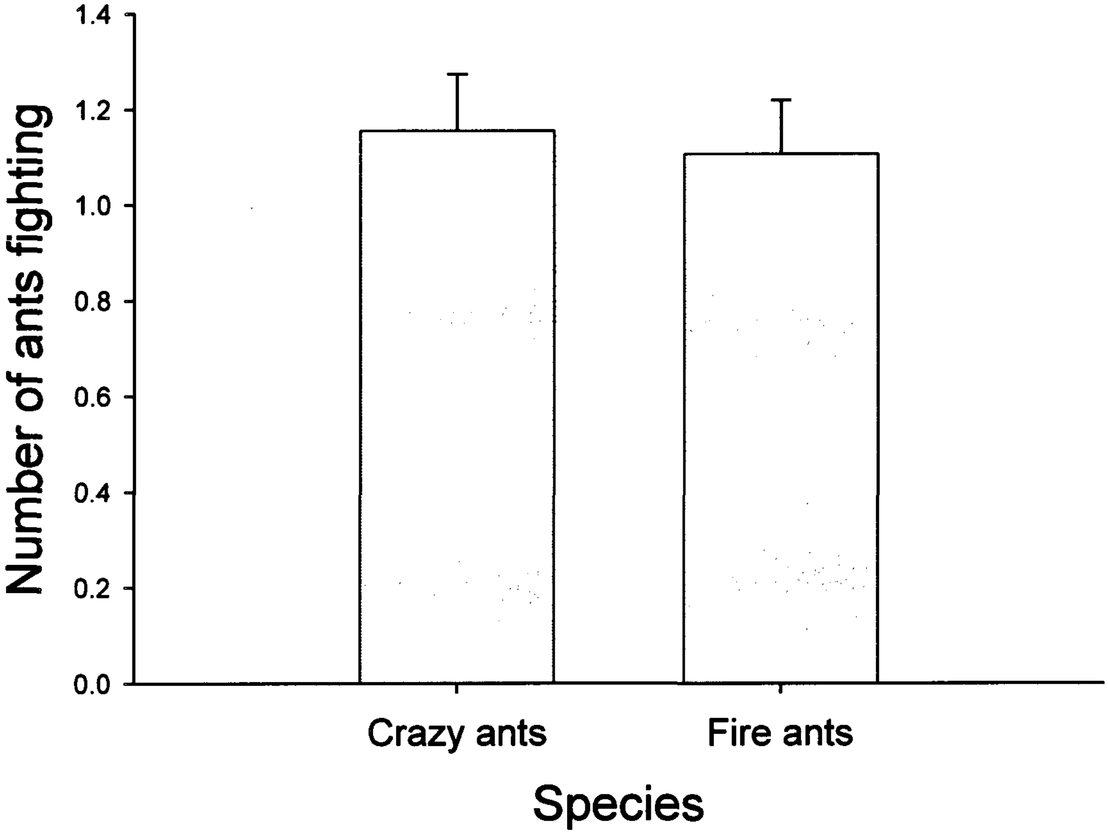


Figure 2.3. The number of dead Rasberry crazy ants and dead fire ants after 1 hour. Crazy ants had higher mortality than fire ants (p = 0.039). The maximum possible mortality was 5 ants. Error bars indicate + 1 SE.

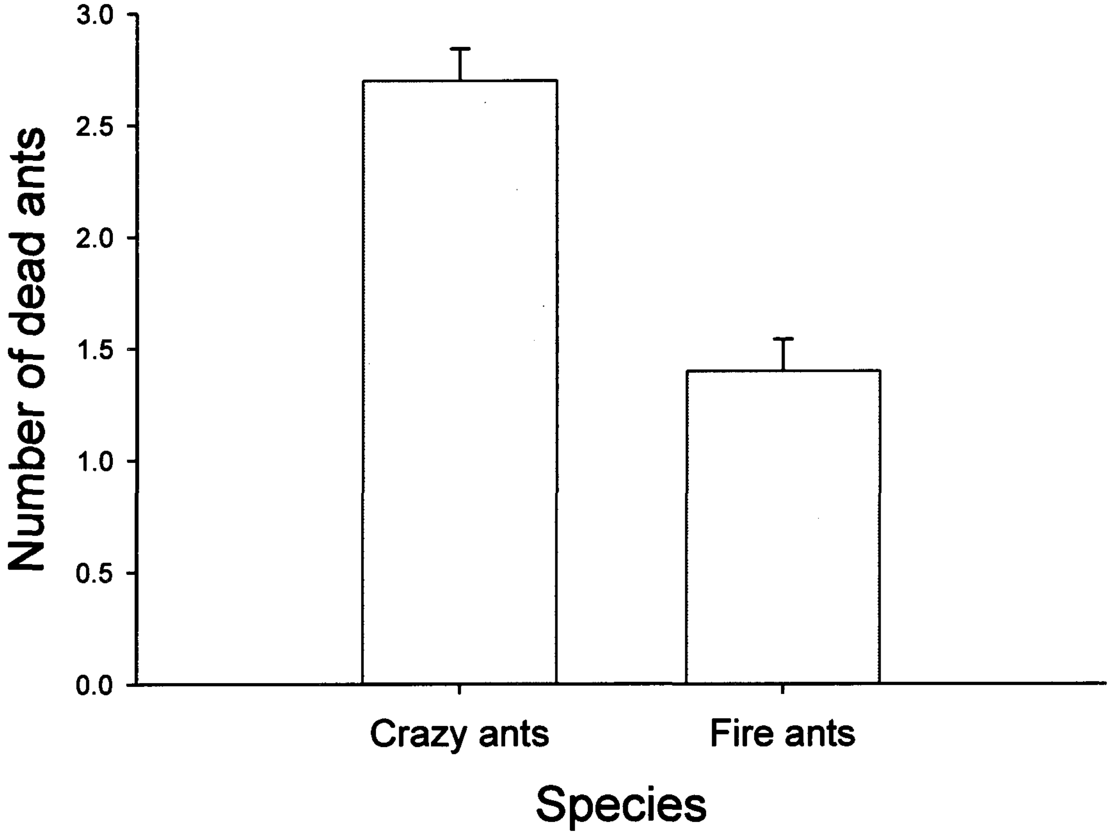
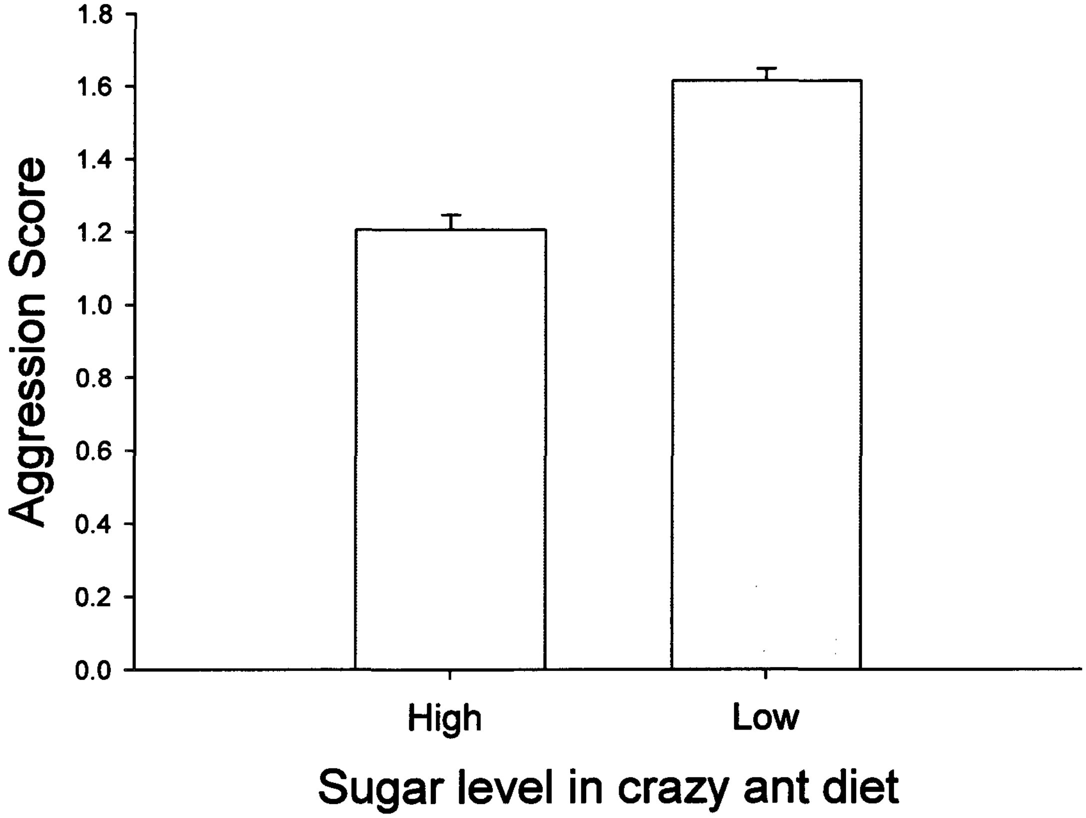


Figure 2.4. The effect of sugar level in crazy ant diet on the average aggression score for each set of fire ants and crazy ants. Aggression scores are assigned according to the scale described by Suarez et al. (1999). Aggression scores for interactions between fire ants and crazy ants were higher when crazy ants received a low sugar diet (p = 0.037). Error bars indicate + 1 SE.



Chapter 3

Colony-Level Competition Between Rasberry Crazy Ants (*Paratrechina* sp. nr. *pubens*) and Red Imported Fire Ants (*Solenopsis invicta*)

Abstract

In further examine whether red imported fire ants (*Solenopsis invicta*) can provide biotic resistance to Rasberry crazy ants (*Paratrechina* sp. nr. *pubens*), I conducted a laboratory experiment and a field experiment that examined colony-level competition between these two species. In the laboratory experiment, colonies of fire ants and crazy ants were standardized by worker number or by biomass and used as either controls or in competing pairs. Control colonies had access to a foraging tray with sugar water and mealworms, while competing colonies shared a foraging tray with a colony of the opposing species. For both colony sizes, and in both control and competition settings, crazy ants recruited more quickly and in higher abundances to baits. Crazy ants also controlled significantly more baits than fire ants did at the end of the experiment. These results suggest that crazy ants, when evenly matched in colony size with fire ants, are capable of discovering baits first and excluding fire ants from resources, thus breaking the dominance-discovery trade-off. In order to simulate the introduction of crazy ants to new areas, I also conducted a field experiment that moved small crazy ant colonies from a heavily invaded site to another location 750m away that had not yet been invaded. I then monitored the ant recruitment to sugar and protein baits for three weeks. Crazy ants discovered baits more quickly, but were subsequently displaced by fire ants, which recruited to both sugar and protein in higher abundances than crazy ants and controlled the majority of baits. Crazy ant recruitment increased through time in crazy ant addition

locations, but fire ant recruitment decreased in both control and crazy ant addition locations, suggesting that crazy ants responded to, rather than caused, drops in fire ant recruitment. Together, these findings suggest that crazy ants may not be able to displace fire ants in areas that cannot support high crazy ant densities, a factor which may restrict the potential spread of crazy ant populations and enable the two invasive species to coexist in a patchy distribution.

Introduction

Colony-level antagonistic interactions are fundamental to the structuring of ant communities (Hölldobler and Wilson 1990). Though aggression assays which focus on worker interactions usually accurately predict whether two colonies will behave aggressively towards one another (Roulston et al. 2003), they do not necessarily predict which colony will have a competitive advantage. In some species, such as the highly invasive Argentine ant (*Linepithema humile*), an individual ant may not be successful in one-on-one interactions with their competitors, but as part of a larger colony, the ants can outcompete and displace competing colonies (Holway 1999, Buczkowski and Bennett 2008). In this study, a laboratory and a field experiment were used to assess the colony-level competitive ability of the non-native Rasberry crazy ant, *Paratrechina* sp. nr. *pubens*. In the lab, Rasberry crazy ants were paired with red imported fire ants, *Solenopsis invicta*, while crazy ant colonies in the field interacted with the existing ant community in the coastal tallgrass prairie of the Texas Gulf Coast, a habitat which is dominated by fire ants. This is the first time that colony-level competitive ability has been assessed in the recently introduced invasive threat, Rasberry crazy ants, and it is the first study, to my knowledge, examining colony-level competition between a long established invasive ant and newly introduced ant species.

Relative colony size has been shown to be important in determining the outcome of competitive interactions between colonies. In Argentine ants, competitive performance increases with colony size in laboratory trials against native ants, and a threshold of relative abundance is necessary for Argentine ants to maintain a dominant worker presence at baits (Holway and Case 2001, Walters and Mackay 2005). Studies with red imported fire ants have also indicated the importance of relative abundance, as competitive success is affected by colony size in trials with native as well as invasive ants (Morrison 2000, Kabashima et al. 2007). Therefore, in the laboratory experiment we paired crazy ants and fire ants in colony units that had been standardized by either biomass or worker number. Additionally, the crazy ant colonies used in the field experiment were smaller than the existing fire ant nests. Together, testing the competitive ability of these different relative abundances of crazy ants may lead to important conclusions about the ability of the Rasberry crazy ant to expand its range in the face of competition from fire ants.

One advantage of a colony-level approach is that is possible to examine recruitment to and dominance of baits, in addition to fighting and mortality. Ant species are often classified as either opportunists, which specialize in discovering baits quickly; extirpators, which arrive to baits later but dominate discovered resources; or insinuators, which inconspicuously thieve food from dominant ants (Wilson 1971). Fellers (1987) first proposed that there may be an evolutionary trade-off between resource discovery and behavioral dominance of resources, a hypothesis known as the dominance-discovery tradeoff. However, more recent studies have shown that some invasive ant species can break this trade-off in their introduced range and can both discover and dominate baits (Davidson 1998). Therefore, examining the colony-level competitive interactions of crazy ants and fire ants both in the laboratory and in the field may lead to new insight into the community role of crazy ants in their introduced range, and more specifically, whether crazy ants break the dominance-discovery trade-off.

Our experimental design allowed us to ask the following questions: 1) Are fire ants or crazy ants more successful in colony-level interactions? 2) Does relative abundance of crazy ants affect the outcome of colony-level interactions? 3) Do crazy ants follow the dominance-discovery tradeoff? and 4) Do crazy ants fill the same role in the dominance-discovery tradeoff in field conditions when they are much less abundant than fire ants? The answers to these questions may lead to a greater understanding of the Rasberry crazy ant introduction, as well as the ability to better predict the future of Rasberry crazy ant populations in their introduced range. More importantly, these questions examine whether fire ants, an well-established invasive species, can provide biotic resistance to the spread of the newly introduced crazy ants.

Methods

Colony-level competition

All ants used in this experiment were from field-collected, polygynous colonies. *Paratrechina* sp. nr. *pubens* colonies were collected in Pearland, TX (29.55°N, 95.28°W) on May 24 and May 31, 2008. *Solenopsis invicta* colonies were collected in Katy, TX (29.93°N, 95.94°W) in early May 2008. After separating ants from the nesting material with which they were collected by flooding the nests, each colony was placed in a 24cm x 11cm plastic nest box. To prevent ants from escaping, nest boxes were ringed with a thin layer of Tanglefoot (Tanglefoot, Grand Rapids, Michigan, USA) near the top of the inside walls. Each colony was fed a standard diet of sugar water daily and half of a freezer-killed mealworm (Armstrong's Cricket Farm, West Monroe, LA) every other day. In order to obtain colonies of uniform size for competitive pairings, field-collected colonies were divided into smaller units from June 18 to July 1, 2008. Each unit contained one queen, no brood, and either 1000 workers in colonies matched by worker number or 500 mg of workers in colonies matched by biomass. Because counting individual workers was not feasible, we used an average per worker mass to extrapolate the mass of 1000 workers. For crazy ants, we randomly selected and weighed between 16 and 26 workers from each of 10 different colonies, recording the number of workers and the total weight each time. For fire ants, we repeated this process with 22 to 28 workers from each of 13 different colonies. The average weight per Rasberry crazy ant was 0.68mg, and the average weight per fire ant was 1.43mg. Therefore for colonies that were matched by worker number, we used 680.0 ± 50.0mg of crazy ants or 1430.0 ± 100.0mg of fire ants.

In total, we created 16 units of crazy ants with 500mg of workers, 16 units of crazy ants with 1000 workers, 15 units of fire ants with 500mg of workers, and 16 units of fire ants with 1000 workers. These colony units were haphazardly assigned to a treatment of either control or competition such that there were 8 control colony units of crazy ants standardized by biomass, 8 control colony units of crazy ants standardized by worker number, 7 control colony units of fire ants standardized by biomass, 8 control colony units of fire ants standardized by worker number, 8 competition pairs of crazy ant and fire ant colony units standardized by biomass, and 8 competition pairs of crazy ants and fire ants standardized by worker number.

For all but two units, the queen and the workers came from the same source colony. The two exceptions were crazy ant units from a source colony with a shortage of queens in relation to the amount of workers. Queens from a different source colony were used, however all of crazy ant source colonies came from a population where the ants behave as a supercolony (Hölldobler and Wilson 1990), and there was no aggression toward the adopted queen. Most (14 of 20) fire ant source colonies were used only once to make a colony unit, though 6 of 20 were used between 2 and 5 times. Each field-collected crazy ant colony was used to make between 2 and 6 colony units. In 5 cases, the same source colony was used to make two colony units for the control treatment with the same standardization factor (500 mg workers or 1000 workers), and in one case the same source colonies were used for 2 competing pairs of fire ants and crazy ants. We treated these colony units and pairs as independent data points because the queen and workers were unique for each unit and a preliminary analysis showed that the response variables of these units were not more similar to each other than to other colony units.

Colony units were housed in 20cm x 12cm x 6cm plastic containers with a layer of Tanglefoot around the upper edge. Each colony unit was allowed to acclimate to its container for 2 to 14 days before the start of the experiment, during which time all colony units continued the diet of sugar water and freezer-killed mealworms. Colony units were then starved for 24 hours before the start of the experiment on July 3, 2008, at which point they were given access to an identical foraging tray via a 5cm-wide bridge of uncoated metal hardware cloth. Each foraging tray contained a freezer-killed mealworm (cut in half) and a 1-ml Eppendorf tube filled with sugar water-soaked cotton placed on top of a 7.6cm x 12.7cm white card. Each colony unit in the control treatment was connected to a unique foraging tray, while each pair of competing colony units shared a foraging tray. The set of hardware cloth bridges attaching competing colonies to the foraging tray also allowed for the movement of ants between nest boxes. Each bait was removed, weighed, and replaced with a fresh bait item on days 3 and 5 of the experiment. The average desiccation of control baits (without ant access) was subtracted from the difference between the initial and final weight of each bait item to determine the mass of bait consumed during the 48-hour period. This calculation method sometimes led to negative values for overall resource consumption.

Behavioral data were collected every 15 minutes for 1 hour and 45 minutes beginning 10 minutes after the bridges were first put in place at 1400h CST on July 3, 2008. Data was then collected each hour until 3 hours and 55 minutes after the start of the experiment and at 24-hour intervals from the start of the experiment until its completion on day 6 (July 8, 2008). On both days 3 and 5, behavioral data were also collected after sugar water and mealworms were replaced, at 51 hours and 10 minutes and 99 hours and 10 minutes after the start of the experiment. During each data collection, the number of ants of each species at the sugar water and at the mealworm was recorded for all colony units. In addition, for paired competition units, we recorded the number of ants of each species involved in fights, as well as the number of ants in the nest box of the competing species. After the last data collection event at 1400h on July 8, 2008, bridges were removed and dead ants were collected from nest boxes and foraging trays and stored in alcohol until they were counted to assess mortality from each colony.

Statistical analysis

All statistical analyses for the colony-level competition experiment were performed in JMP 7.0.2 (SAS Institute Inc., Cary, NC, 2007). The following response variables were used in analyses for each colony or pair of colonies (in the case of resource consumption): mortality at the end of the experiment (number of dead ants not stuck in Tanglefoot), mealworm and sugar water consumption (averaged across the 3 pieces of each bait given to each colony), total recruitment across all time periods and time until recruitment (when 2 or more ants of the same species were present) for each bait type, the total number of ants fighting and the total number of ants in the other species' nest box summed across all observations, and final bait control (the species present on each bait type after food was replaced on day 5). We tested for correlations between all response variables. For all variables other than bait dominance, the data were analyzed as follows: control colonies were compared using ANOVA's with species, standardizing factor (biomass or worker numbers), and the interaction between the two as factors. Post-hoc analyses on significant interactions were conducted using Tukey's HSD tests. To compare between species in the competition treatment, the difference between the two species was calculated and the mean was tested for significant deviation from 0 using a 1-group, 2-tailed t-test. The effect of standardizing factor was analyzed using a one-way ANOVA. The following transformations were performed to meet assumptions of normality: mealworm consumption for control colonies and total ants fighting were square root transformed, and mortality and mealworm and sugar water recruitment were

$\log_{10}$ transformed. Three outlying data points were excluded from analysis: one crazy ant control colony for sugar water consumption, one crazy ant control colony for mortality, and 1 competing pair of colonies for total ants in the other species nest box. When transformations to normality were not possible, Wilcoxon signed rank tests were performed for each factor (instead of an ANOVA) or mean difference (instead of a 2-tailed t-test). The specific test used for each response variable is shown in Table 3.1. Bait dominance was analyzed for a species effect using binomial probability.

Crazy ant addition field experiment

Field research was conducted at the University of Houston Coastal Center (UHCC) in La Marque, TX (29.38°N, 95.04°W). The 400-hectare property has approximately 80 hectares of undisturbed native prairie, with the remainder of the property composed primarily of non-native woody species (especially Chinese tallow tree [*Triadica sebifera*]). In April 2007, Rasberry crazy ants were found in high abundances in the southwest corner of the UHCC property. It is unknown how the ants were introduced to UHCC; they may have arrived with a load of contaminated fill dirt, or they may have spread from a possible infestation at the landfill on the western border of the UHCC property. In the infested area, nests were located in the woods, but foraging ants were readily apparent in open spaces, including roadways and prairie. The population appeared to be unicolonial, with no noticeable aggression between nests (Hölldobler and Wilson 1990).

Because the high-density infestation was limited to the southwest corner of the property, we were able to locate an area without a high abundance of crazy ants in the southeast corner of UHCC in a prairie bordered by woods, approximately 750m from the infestation. We selected 24 locations at the edge of the prairie and the woods, each 25m apart. Each location was randomly assigned to either a crazy ant colony addition treatment or control. Prior to establishing treatments, we baited for ants on June 17, 2009, following the same protocol used in all subsequent baitings. At each location, we placed two 9cm diameter Petri dishes; one dish contained a ~2gm slice of Libby's Vienna Sausage, and the other dish held a cotton ball soaked with 30% honey water. We monitored each Petri dish for 10 minutes or until it was discovered, at which point we recorded the ant species and the number of minutes that had passed since opening the bait. One hour after baits were opened, we closed the Petri dishes and placed them in a freezer until the number of each species in each dish could be recorded.

Five ant species were collected during the initial baiting: *Solenopsis invicta* (17 dishes), *P.* sp. nr. *pubens* (8 dishes), *Solenopsis* sp. cf. *geminata* (3 dishes), *Crematogaster* sp. (1 dish), and *Pheidole* sp. (1 dish). *Solenopsis invicta* was the most commonly collected ant species and had the highest average recruitment with 153.2 ants per dish. Crazy ants recruited to a total of 7 locations: to sugar baits at 5 locations, to protein at 1 location, and to both sugar and protein at 1 location. The average crazy ant recruitment was 25 ants, and all but the 2 protein baits had 12 or fewer crazy ants.

On June 19, 2009, 12 crazy ant nests were collected in decaying logs from the high-density infestation area. Collection of superficially similar nests from a different site in 2008 revealed that these nests often contained more than 3000 workers and at least 5 queens (personal observation, K. Horn). Therefore, while we were unable to measure the size of the experimental colonies, each colony likely had at least 1000 workers and more than 2 queens. Because the nests had been collected from a wooded area, we placed a nest of crazy ants 1.5m away from the edge of the prairie into the woods at each location assigned to the crazy ant addition treatment. At each control location, we placed a similar amount of ant-free decaying wood 1.5m into the woods. After the treatments were established, ant baiting was conducted on June 24, June 29, July 3, July 8, July 14, and July 29 which was 5, 10, 14, 19, 25, and 40 days after colony additions.

Statistical analyses

Crazy ants and fire ants were the only two species that recruited to baits with regularity and abundance, therefore the analyses focused on these two species. On all days except July 29, baiting data revealed higher average crazy ant recruitment at crazy ant addition locations than at control locations. Therefore, because the treatment was not reflected in census data on July 29, that date was excluded from analysis. Repeated measures ANOVA's performed in StatView 5.0 (SAS Institute Inc., Cary, NC, 1998) were used to determine if crazy ant and fire ant recruitment to baits after 1 hour changed through time at control and crazy ant addition locations using the sampling dates from June 24 to July 14 (i.e. after crazy ants were added). These ANOVA's included treatment and bait type, as well as all possible interactions, as factors. Using presence and absence at baits after 1 hour on July 8 and July 14, we used 2 contingency tables for each bait type to determine if a single ant species dominated baits more often than 2 or more ant species shared baits. To examine the number of baits discovered within 10 minutes, we used a series of 2x2 contingency tables tested for significance in Microsoft Excel or in JMP 7.0.2. Specifically, we tested whether the number of baits discovered in 10 minutes differed by bait type, species, or treatment. We also tested whether average discovery time differed by species, bait type, treatment, or any of the interactions of those variables using an ANOVA in SAS 9.1 (SAS Institute Inc., Cary, NC, 2003). Because only 5-10 of 48 baits were discovered within 10 minutes on any given day, all dates were summed for contingency tables and recruitment time analysis. None of the locations had repeated discoveries of sugar baits, but 10 out of 19 locations that discovered protein dishes had repeated discoveries. Though repeated discoveries of baits on different days are not truly independent data points, this was the only way to conduct meaningful statistical tests.

Results

Colony-level competition

For control colonies, ant species was often a significant factor. Crazy ants had significantly lower mortality than fire ants (Table 3.1, Figure 3.1) and consumed significantly less sugar water (Table 3.1, Figure 3.2), but there was no difference between species in mealworm consumption (Table 3.1). Crazy ants recruited in greater numbers than fire ants to mealworms but not to sugar water (Table 3.1, Figure 3.3). Crazy ants also discovered and recruited to both mealworms and sugar water more quickly than fire ants did (Table 3.1, Figure 3.4).

Standardizing factor (mass versus number of workers) generally did not significantly affect response variables for control colonies (Table 3.1). The exception was that mealworm and sugar water total recruitment were both higher for colony units standardized by worker number (worker numbers were higher for colonies standardized by worker number than by biomass). The interaction between species and standardizing factor was significant only for total recruitment to sugar water (Table 3.1). For sugar water, recruitment was significantly higher for both species in the worker number treatment than for fire ants in the biomass treatment, but not significantly higher than crazy ants in the biomass treatment. The sugar water recruitment in the biomass treatment was not significantly different for the two species.

For competition colonies, species was again frequently a significant predictor. Differences in recruitment abundances and recruitment and discovery times were also strongly affected by species identity, as crazy ants had a higher total recruitment to both sugar water and mealworms and recruited to sugar water and mealworms more quickly than did fire ants (Table 3.1, Figures 3.3 and 3.4). Crazy ants also had higher numbers of ants engaged in fights and in the other species' nest box than did fire ants (Table 3.1, Figure 3.5). However, mortality was not different between the two species in competition (Table 3.1, Figure 3.1). Crazy ants were the only species at baits at the end of the experiment significantly more often than fire ants (Table 3.1). Standardizing factor was not significant for any of the response variables for competition colonies (Table 3.1).

All significant correlations between response variables are shown in Table 3.2. In general, discovery and recruitment times between and within bait types were positively correlated, and sugar water and mealworm recruitment abundances were positively correlated. Sugar water and mealworm recruitment times were negatively correlated with the recruitment abundances for these baits. Mealworm recruitment time was also negatively correlated with mortality.

Crazy ant addition field experiment

On average, crazy ant recruitment increased through time, but not significantly so ($F_{4,176} = 1.683$, $p = 0.1559$, Figure 3.6). There was a trend for crazy ant recruitment to be higher in crazy ant addition locations than control locations ($F_{1,44} = 3.697$, $p = 0.0610$), and crazy ant recruitment was higher for protein than for sugar ($F_{1,44} = 6.770$, $p = 0.0126$, Figure 3.7). The interaction between bait type and treatment was sizeable but not significant ($F_{1,44} = 2.825$, $p = 0.0990$). There were no significant interactions with time and any other factors. Fire ant recruitment decreased significantly through time ($F_{4,176} = 5.154$, $p = 0.0006$, Figure 3.6), but recruitment was not affected by treatment ($F_{1,44} = 0.026$, $p = 0.8718$). Recruitment to protein baits was much higher than recruitment to sugar baits ($F_{1,44} = 22.817$, $p < 0.0001$, Figure 3.7). On average, per bait recruitment was higher for fire ants than for crazy ants (Figure 3.6). Fire ants also controlled significantly more protein baits ($p = 0.0394$) but not sugar baits ($p = 0.1444$) after 1 hour (on July 8 and 14). On both July 8 and July 14, there were no instances of crazy ants and fire ants co-occurring at a protein bait ($\chi = 10.1931$, $p = 0.0006$), yet there was no evidence of sugar bait exclusion by either species ($\chi = 0.9470$, $p = 0.9538$).

Summing all time periods, crazy ants discovered 16 of the baits in 10 minutes, and fire ants discovered 15 of the baits. Protein baits were discovered in 10 minutes significantly more often than sugar baits, regardless of species ($\chi = 11.242$, $p = 0.0008$). Fire ants and crazy ants did not differ in the amount of protein baits ($p = 0.1489$) or sugar baits ($p = 0.2734$) they discovered. Protein baits were discovered more often than sugar baits by both crazy ants ($\chi = 7.185$, $p = 0.0074$) and fire ants ($\chi = 3.615$, $p = 0.0573$). Treatments did not affect the number of baits discovered by fire ants ($\chi = 1.810$, $p = 0.1785$) or by crazy ants ($\chi = 2.467$, $p = 0.1163$). Similarly, treatment had no effect on average discovery time ($F_{1,34} = 0.26$, $p = 0.6148$) for the two species, and the treatment by species interaction was insignificant ($F_{1,34} = 0.03$, $p = 0.8742$). In contrast, bait type significantly affected discovery time ($F_{1,34} = 6.71$, $p = 0.0140$), with sugar baits discovered more quickly than protein baits (Figure 3.8). Species was also a significant factor ($F_{1,34} = 7.62$, $p = 0.0092$), as crazy ants discovered baits more quickly than fire ants (Figure 3.8).

Discussion

The results of the colony-level competition experiment indicate that crazy ants recruit to baits more quickly than fire ants both in isolation and in competition (Figure 3.4). However, despite their quick recruitment, they do not consume more resources than fire ants in control colonies (Table 3.1). In fact, the only significant difference between species in resource consumption was for sugar water consumption, of which fire ants consumed more (Figure 3.2). Yet, crazy ants were the species found most often at both bait types at the end of the experiment in competition colonies (Table 3.1), suggesting that their recruitment times and abundances may be important in maintaining access to baits when faced with a competitor.

In general, significant differences between species in controls remained significant for the same response variables in competition colonies (Table 3.1). Crazy ants recruited more quickly to mealworms and sugar water and in higher numbers to mealworms than fire ants did in both control and competition colonies (Figures 3.3 and 3.4). In fact, the crazy ant advantage was larger in competition than in control. This finding suggests that crazy ants, which have an inherent advantage over fire ants in recruitment speed and numbers, may have deterred fire ants from discovering and recruiting to baits in competition. Another result which points to this conclusion is that there was no difference in sugar water recruitment between species in control colonies, but crazy ants recruited in higher numbers than fire ants in competition colonies (Table 3.1). In contrast to this finding, crazy ants had significantly less mortality than fire ants in control colonies, but not in competition colonies (Table 3.1, Figure 3.1). This suggests that fire ants, which experienced heavy mortality in control colonies, were able to inflict a lot of casualties on crazy ants. Nevertheless, crazy ants had significantly more workers involved in fights and in the nest box of their competitor than fire ants did (Figure 3.5), but they did not have significantly more mortality than fire ants. The high aggression but low mortality for crazy ants is similar to results of aggression assays using 5 workers of each species (see Chapter 2) and reinforces the findings that aggressive interactions do not correlate with crazy ant mortality.

Standardizing factor (worker number or biomass) did not significantly affect most responsible variables, including mortality, resource consumption, and number of ants fighting (Table 3.1). The one exception was that colonies standardized by biomass, which had fewer workers than colonies standardized by worker number, had fewer ants recruiting to both bait types. Additionally, the lack of significant interactions between species and standardizing factor suggest that a slight crazy ant numerical advantage does not affect crazy ant recruitment and survival when compared to or competing with fire ants. In contrast, Morrison (2000) found that *S. invicta* controlled more baits and inflicted more mortality on the native fire ant, *S. geminata*, when colonies were paired by biomass rather than worker number. *Solenopsis geminata* workers are much larger than *S. invicta* workers (1010 workers per gram compared to 1720 workers per gram), therefore pairing by biomass gave *S. invicta* a numerical advantage. However neither biomass nor worker number pairings gave red imported fire ants a numerical advantage over crazy ants, as crazy ants are much smaller than fire ants, which may explain why fire ants performed more poorly than crazy ants in both colony unit types. Nevertheless, the results of the laboratory study, including the finding that standardizing factor was insignificant, suggest that if a crazy ant population is similar in number to a neighboring fire ant population, the crazy ants may be able to outcompete fire ants for resources, even if the fire ants have a greater population biomass than the crazy ants.

The laboratory findings on recruitment times mirror the results of the field experiment, as crazy ants discovered both bait types more quickly than fire ants in the field (Figure 3.8). In contrast to the laboratory findings, crazy ant recruitment to both bait types was much lower than fire ant recruitment (Figure 3.7), and this may be due to the large difference in field abundance between the species. Relatively small colonies of crazy ants were introduced to an area dominated by fire ants nests, which likely each have thousands of workers (Markin et al. 1973). The difference in recruitment abundance appears to be more important than recruitment time in determining control of baits, as fire ants controlled significantly more protein baits (which received the highest recruitment by both species) at the end of 1 hour. Also, in the field, ants of both species recruited to protein in higher numbers, though no preference for protein was shown in the laboratory experiment (Figure 3.7). This may be because laboratory colonies were fed a diet that included generous amounts of protein until 24 hours before the start of the experiment, whereas field colonies were likely protein-limited, as ants are often more abundant than prey items (Tobin 1991, 1994). Also, laboratory colonies had negligible brood, whereas field colonies presumably had brood, which have a greater need for protein than adult workers (Hölldobler and Wilson 1990). Ant species did not co-occur at protein baits, suggesting that protein resources, even when available, may be difficult to control in the field due to high levels of competition, thus increasing a colony's need to recruit in high numbers.

In a field setting, crazy ants discover baits more quickly but fire ants dominate baits after 1 hour. Thus, in this context, crazy ants would be considered opportunists, who discover food quickly and exploit it before other ants arrive, while fire ants would be considered extirpators that aggressively dominate discovered resources (Wilson 1971). Both species fit the dominance-discovery tradeoff model in the field setting (Fellers 1987). However, in the laboratory experiment, crazy ants discovered baits more quickly and controlled more baits than fire ants did at the end of the experiment. This finding suggests that when crazy ant colonies are evenly matched in worker number or biomass with fire ants, crazy ants may be both the discoverers and the controllers of baits. Therefore, crazy ants may have a similar community role as Argentine ants, which have also shown exploitative competitive ability as well as behavioral dominance in part of their invasive range (Holway 1999). Nevertheless, ant community roles, including those of opportunists and extirpators, can be context-dependent (Morrison 1996), and our findings are an example of this phenomenon, as crazy ant abundance appears to be important in determining their role as opportunists or extirpators. Our findings are similar to that of Walters and Mackay (2005), who showed that Argentine ants were able to overcome the native ant *Iridomyrmex 'rufoniger'* in laboratory trials only when Argentine ant colonies were 5 to 10 times larger than *Iridomyrmex* colonies. Holway and Case (2001) also showed the importance of colony size on exploitative and interference ability in Argentine ants, as every measure of competitive performance increased with worker number. As shown in Chapter 2, fire ants are more successful than crazy ants in individual interactions, yet our findings in the laboratory experiment show that crazy ants can be more successful at a colony level when colony units are closely matched by biomass or worker number. Our field results reinforce the importance of relative crazy ant abundance, as crazy ants recruited to resources in much lower numbers than fire ants and behaved as opportunists, rather than controlling the baits which they discovered first.

In the field experiment, fire ant recruitment decreased in both control and crazy ant addition locations (Figure 3.6). Though crazy ant recruitment increased through time in the crazy ant addition treatment, our data suggest that the decrease in fire ant recruitment is not due to the introduction of crazy ants. Simply, the addition of crazy ant colonies did not cause an immediate and effective displacement of fire ants. Fire ant decline may have been due to environmental factors such as the drought and extreme heat in the weeks prior to and during the duration of the field experiment, when it rained only once. In a laboratory and field experiments, fire ant foraging and bait retrieval has been shown to decrease in increasing temperatures (Drees et al. 2007, Zheng et al. 2007).

Field temperatures often exceeded 35°C in June and July, which may have contributed to the decrease in fire ant recruitment during the experiment. Additionally, fire ants have been found to have higher summer mortality (personal communication, M. Eubanks). Therefore, crazy ants, which have been reported to increase in abundance during the summer months (personal observation, T. Rasberry), are likely responding to, rather than driving, a decrease in fire ant recruitment. Reports in the popular press (such as Blumenthal 2008) that crazy ants rapidly displace fire ants are likely not true. The pattern of a switch from fire ant dominance to crazy ant dominance appears to be correct but the proposed mechanism is not. One possible explanation for the lack of fire ant replacement by crazy ants is that crazy ant colonies used in crazy ant addition locations may not have been large enough to send enough workers to baits to deter fire ants. However, retesting with larger colonies of crazy ants would be difficult due to the logistical and ethical considerations that must be a part of site selection.

Crazy ants have been shown to be less effective fighters in individual contests (see Chapter 2), but they did not differ from fire ants in mortality in competing colonies, and they recruited to baits more quickly and more abundantly than fire ants. Therefore, fire ants do not have a universal competitive advantage over crazy ants. The ability of crazy ants to defend food resources from fire ants may be related to both numerical abundance as well as habitat. Environmental conditions, including soil moisture and stream flow, have been shown to affect the spread of Argentine ants (Holway 1998, Menke et al. 2007), so it is not unlikely that environmental condition could affect the distribution of crazy ants. Even extremely large supercolonies may not be able spread out of the woods, as open spaces adjacent to heavily invaded woody areas generally have low to moderate amounts of crazy ants (personal observartion, K. Horn). Abandoned fire ant mounds, but no active mounds, can sometimes be found in woods heavily invaded by crazy ants, suggesting that crazy ants may have displaced fire ants in those areas but we cannot confirm this pattern from our experiments. Regardless, active fire ant mounds are not found in wooded areas with high crazy ant abundances, suggesting that at the very least, crazy ants are preventing re-establishment of fire ants in a formerly suitable habitat. These clues suggest that in addition to an increased competitive ability with increasing abundances, crazy ants may be competitively superior to fire ants in woody habitats where they manage to become established, but environmental conditions may prevent them achieving high population densities and displacing fire ants in open areas. Though further studies would need to be conducted to confirm this hypothesis, there is an assortment of features of woody habitats that could be important to crazy ant success, such as thermal conditions, litter structure, and availability of sugar resources like honeydew produced by sap-feeding insects. Sugar availability in particular may be important in determining suitable habitat for crazy ants. Argentine ants, which display many characteristics similar to crazy ants as previously noted, were found to establish nests in areas where they had not previously been able to invade following the addition of sucrose feeding stations (Rowles and Silverman 2009). In the case of crazy ants, woody habitats may provide the environmental conditions and resource availability necessary to achieve high densities. A large-scale survey of sites invaded by crazy ants as well as studies examining interactions between fire ants and crazy ants at invasion fronts would be useful in experimentally testing this hypothesis.

Clearly, the competitive abilities of ants are not tested before they are introduced to a new range, yet for new introductions that cannot be eradicated, studies examining competitive interactions between introduced ants and established ants in the introduced range may be useful. Such studies could help assess the potential invasive risk associated with different species. In some scenarios, this could aid in the development of a management plan, whether it be active control of a high risk species, or a more passive approach for species which are less competitively dominant and may not require the use of finite resources reserved for invasive species control. Our findings suggest that colony size and habitat type may be key factors in the crazy ant invasion. Crazy ants can coexist with fire ants in low abundances in open habitats (as shown with pre-crazy ant addition field baiting results), yet observations have shown high abundances of crazy ants and an absence of fire ants in invaded woody areas. Crazy ants are more successful in clashes with fire ants on a colony- rather than an individual-level, yet relative abundance alone cannot explain the invasion dynamics, as open areas adjacent to heavily invaded woodlands are often nearly absent of crazy ants. Further studies examining invasion front dynamics may be useful in testing this hypothesis, yet our findings are a key preliminary step that suggests that crazy ant invasions may be restricted to wooded areas and areas free of fire ants, as they are competitively excluded by fire ants in open areas.

Beyond the implications for Rasberry crazy ant management, this study has important implications for the field of invasion biology. As the first study to experimentally examine biotic resistance to a newly introduced species by an established invasive species, these findings demonstrate the importance of competitive interactions between invasive species in the introduced range, which have been largely ignored (but see Belote and Weltzin 2006 and Griffen et al. 2008). The results of this study give compelling reason for further study of antagonistic interaction between introduced species, as research in this area may help not only with species-specific applied issues, but also with our understanding of invasion biology as a whole.

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Table 3.1. Tests for each response variable for each comparison. P< 0.05 are in **bold**.

Factor = standardizing factor (biomass or worker number).

Mortality				
Controls	ANOVA	Species	$F_{1,26}=5.66$	**p=0.0250**
		Factor	$F_{1,26}=1.91$	p=0.1792
		S*F	$F_{1,26}=1.44$	p=0.2413
Competition (RCA-RIFA)	ANOVA	Factor	$F_{1,14}=2.10$	p=0.1696
	t-test	mean=0	$t_{15}=--1.49$	p=0.1581
Mealworm consumed				
Controls	ANOVA	Species	$F_{1,27}=2.37$	p=0.1357
		Factor	$F_{1,27}=0.0001$	p=0.9912
		S*F	$F_{1,27}=0.56$	p=0.4600
Competition	Wilcoxon	Factor	$Z_1=1.00$	p=0.3184
Sugar water consumed				
Controls	ANOVA	Species	$F_{1,26}=5.72$	**p=0.0243**
		Factor	$F_{1,26}=0.52$	p=0.4777
		S*F	$F_{1,26}=0.02$	p=0.9014
Competition	ANOVA	Factor	$F_{1,14}=0.26$	p=0.6175
Mealworm total recruitment				
Controls	ANOVA	Species	$F_{1,27}=6.81$	**p=0.0146**
		Factor	$F_{1,27}=4.72$	**p=0.0387**
		S*F	$F_{1,27}=1.50$	p=0.2317
Competition (RCA-RIFA)	ANOVA	Factor	$F_{1,14}=0.11$	p=0.7409
	t-test	mean=0	$t_{15}=3.35$	**p=0.0044**
Sugar water total recruitment				
Controls	ANOVA	Species	$F_{1,27}=0.29$	p=0.2917
		Factor	$F_{1,27}=0.002$	**p=0.0024**
		S*F	$F_{1,27}=0.03$	**p=0.0299**
Competition (RCA-RIFA)	ANOVA	Factor	$F_{1,14}=0.04$	p=0.8484
	t-test	mean=0	$t_{15}=4.27$	**p=0.0007**
Mealworm recruitment time				
Controls	Wilcoxon	Species	$Z_1=3.63$	**p=0.0003**
Competition (RCA-RIFA)	Wilcoxon	Factor	$Z_1=0.37$	p=0.7120
	Wilcoxon	mean=0	$Z_{15}=-51.00$	**p=0.0019**
Sugar water recruitment time				
Controls	Wilcoxon	Species	$Z_1=2.68$	**p=0.0074**
Competition (RCA-RIFA)	Wilcoxon	Factor	$Z_1=-0.37$	p=0.7101
	Wilcoxon	mean=0	$Z_{15}=-36.00$	**p=0.0024**
Total ants fighting				
Competition (RCA-RIFA)	ANOVA	Factor	$F_{1,14}=1.08$	p=0.3159
	t-test	mean=0	$t_{15}=5.37$	**p<0.0001**
Total ants in other nest box				
Competition (RCA-RIFA)	Wilcoxon	Factor	$Z_1=-1.19$	p=0.2330
	Wilcoxon	mean=0	$Z_7=18.00$	**p=0.0078**
Final bait control				
Competition	Binomial	Species		**p=0.0327**

Table 3.2. Significant correlations between response variables. All correlations with discovery and recruitment times were non-normal, and therefore the correlation value is Spearman's ρ. The Benjamini and Hochberg FDR method (Benjamini and Hochberg 1995) was used to correct for multiple comparisons. MW = mealworm and SW = sugar water.

Variable 1	Variable 2	Correlation	p-value
SW recruitment	MW recruitment	0.4977	0.0053
SW recruitment time	MW recruitment	-0.6936	<0.0001
SW recruitment time	SW recruitment	-0.5298	0.0031
MW recruitment time	MW recruitment	-0.4964	0.0116
MW recruitment time	Mortality	0.4008	0.0471
MW recruitment time	SW recruitment time	0.8359	<0.0001

Figure 3.1. Number of dead ants at the end of the colony-level competition experiment. Fire ants (RIFA) experienced higher mortality than crazy ants (RCA) in control colonies (p = 0.0250), but not in competition colonies (p = 0.1696). Because the standardizing factor affected the total number of ants in a colony unit, the number of dead ants for each species and treatment is divided into worker number colony units and worker biomass colony units. Error bars are +1 SE.

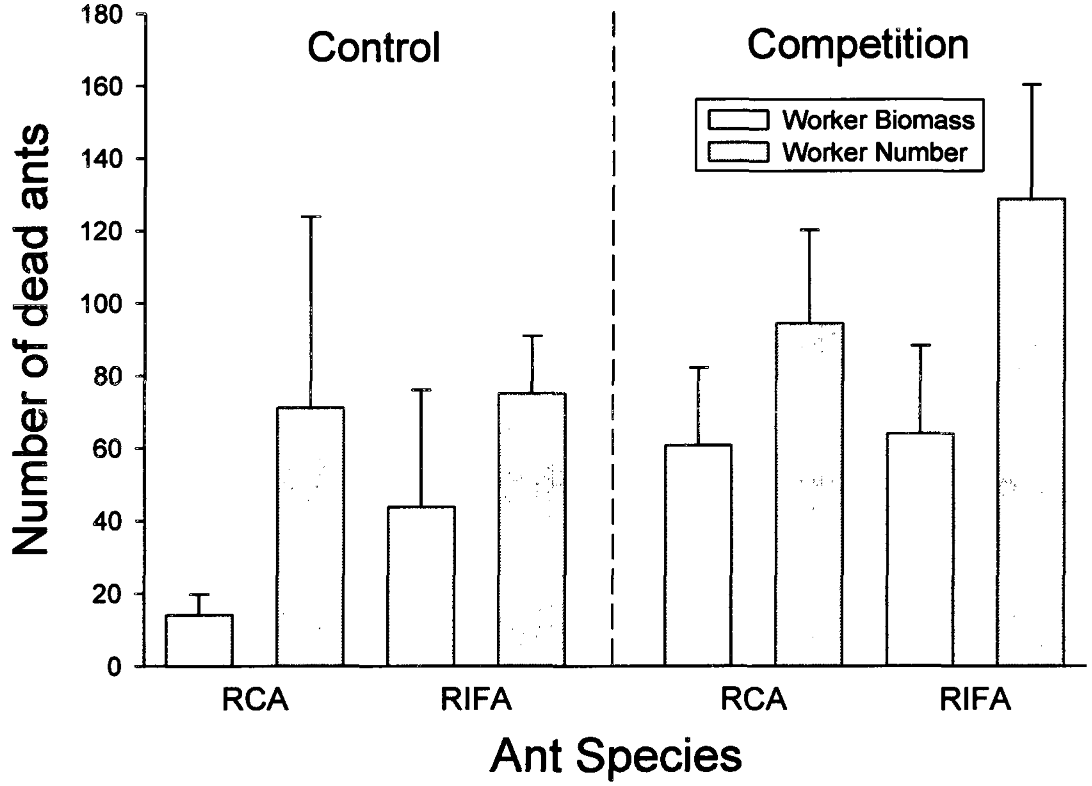

Figure 3.2. Average sugar water consumption by species, adjusted for average desiccation. Fire ants (RIFA) consumed more sugar water than did crazy ants (RCA, p = 0.0243). Average crazy ant sugar water consumption was negative due to the method of calculation (described in detail in the Methods). Error bars are ±1 SE.

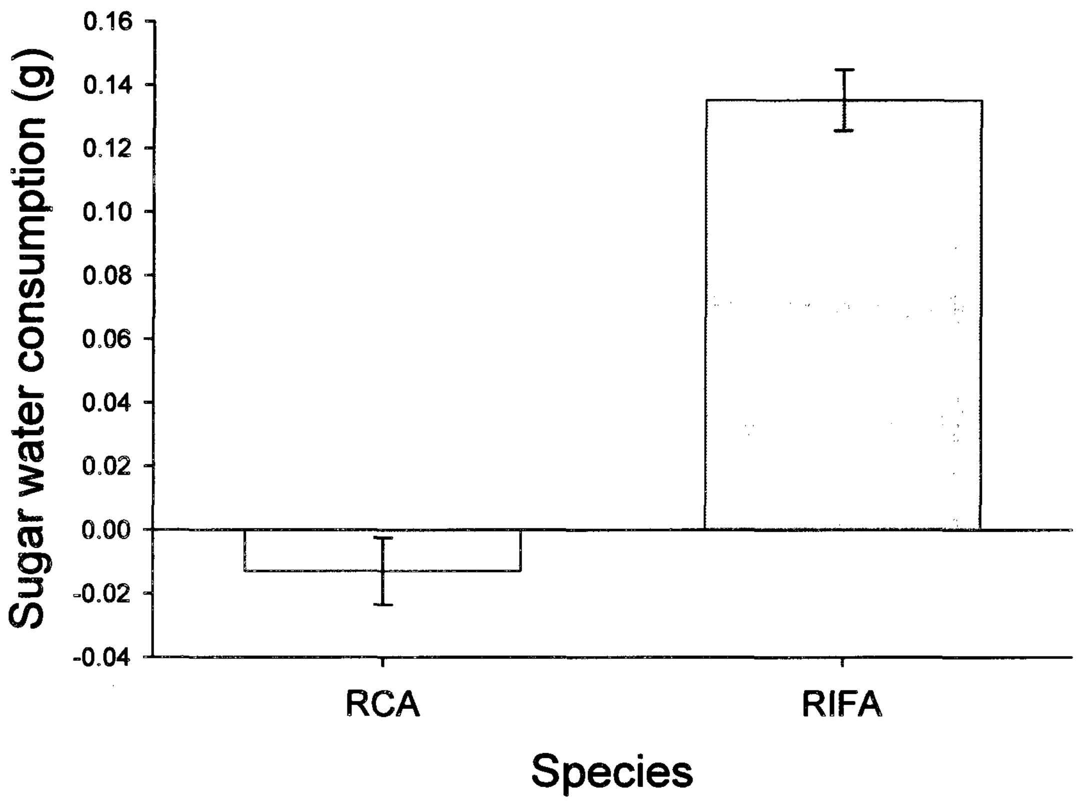

Figure 3.3. Recruitment to mealworms and sugar water. Crazy ants (RCA) recruited to mealworms, but not sugar water, in higher number than fire ants (RIFA) in control colonies (p = 0.0146 and p = 0.2917, respectively). Crazy ants recruited to both baits in higher abundances than fire ants in competition colonies (p = 0.0044 for mealworm, p = 0.0007 for sugar water). Error bars are +1 SE.

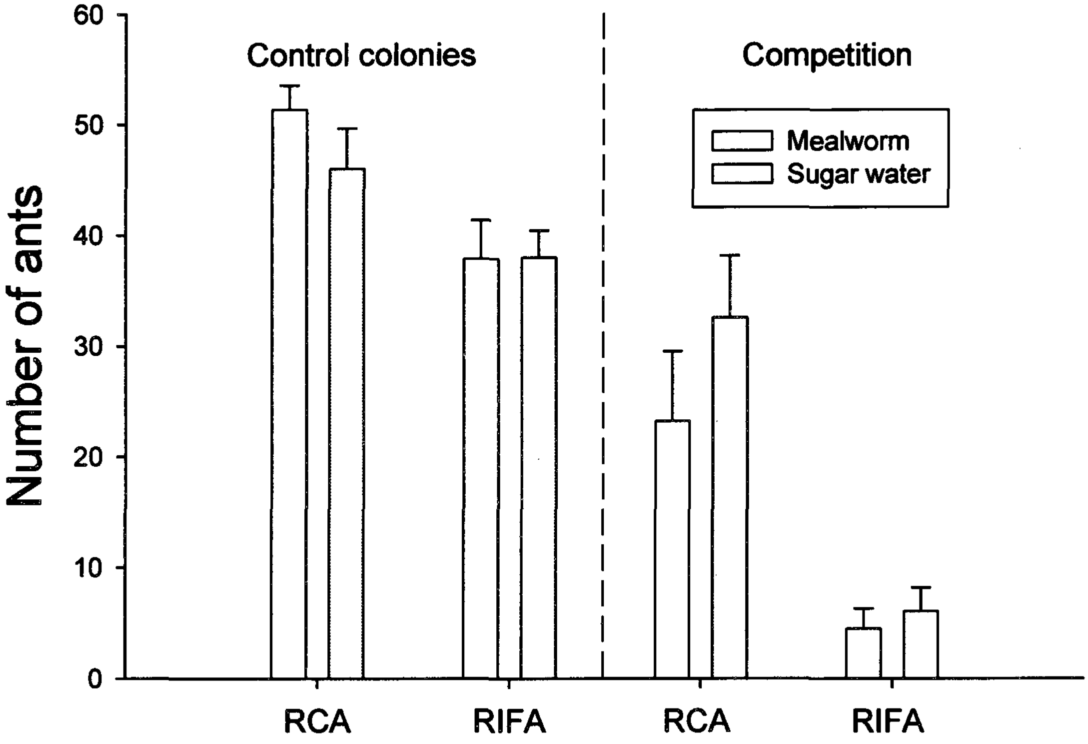

Figure 3.4. Mealworm and sugar water recruitment times. Crazy ants (RCA) recruited to mealworms and sugar water more quickly than fire ants (RIFA) in control colonies (p = 0.0003 for mealworm, p = 0.0074 for sugar water), as well as in competition colonies (p = 0.0019 for mealworm, p = 0.0024 for sugar water). Error bars are +1 SE.

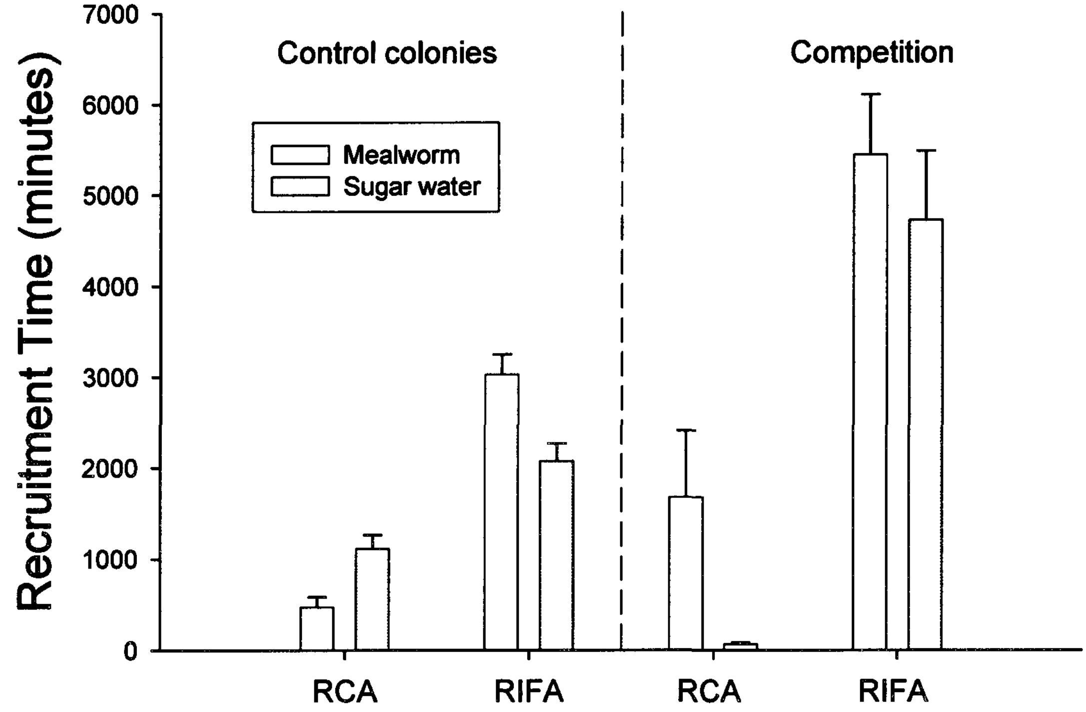

Figure 3.5. Ants fighting and ants in the other nest box. Crazy ants had more ants fighting ($p < 0.0001$) and more ants in their competitors' nest box ($p = 0.0078$) than fire ants did.

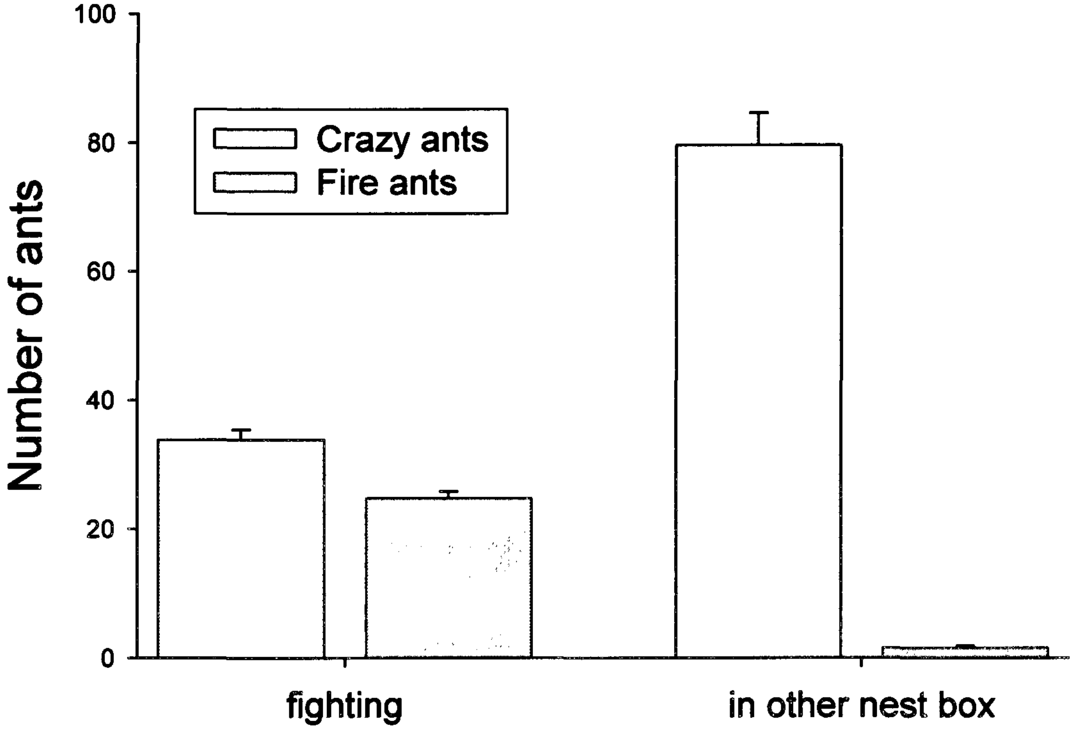

Figure 3.6. Changes in ant recruitment to field baits through time. Sampling began on June 24, five days after treatment establishment, and ended on July 14, twenty-five days after treatment establishment.

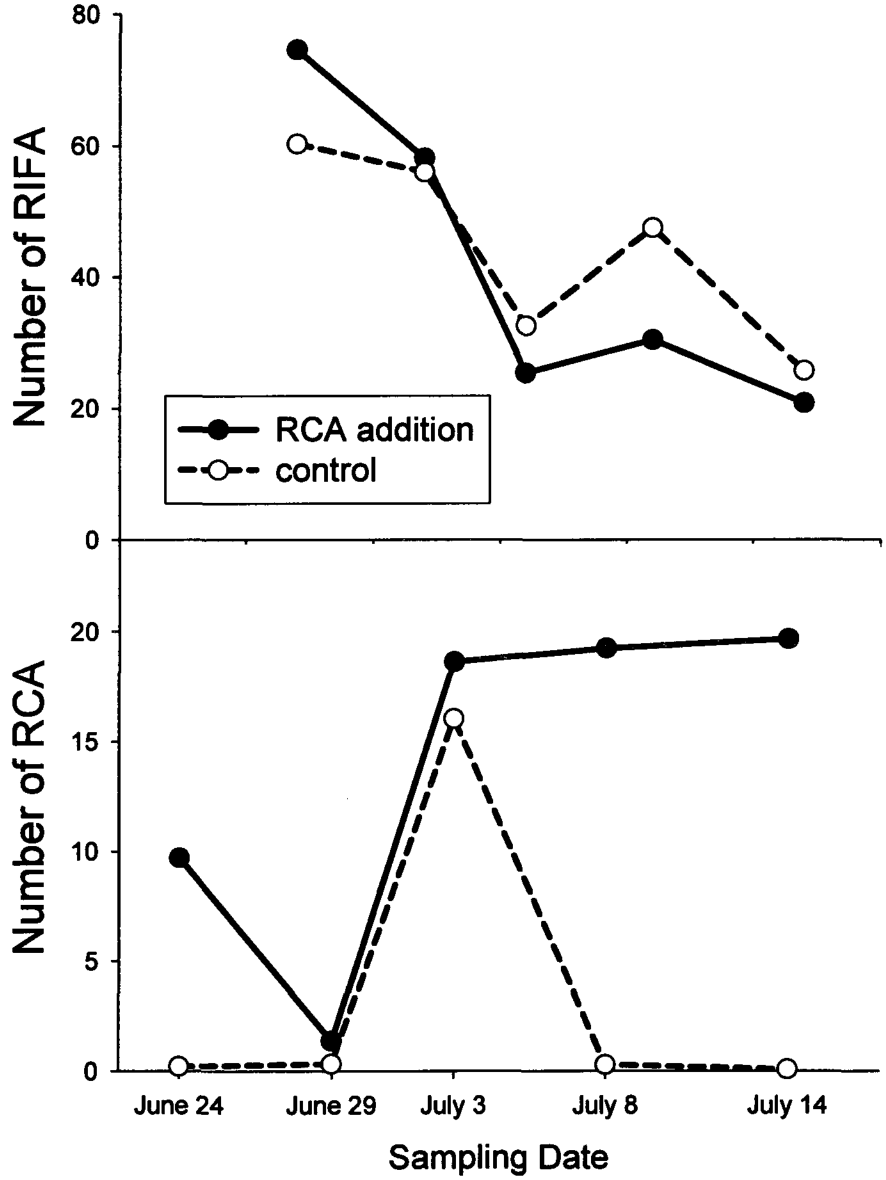

Figure 3.7. Average ant recruitment to field baits by treatment and bait type from June 24 to July 14. Recruitment was significantly higher to protein than to sugar for crazy ants (p = 0.0126) and for fire ants (p < 0.0001). On average, crazy ant recruitment was higher in crazy ant addition locations (p = 0.0610), but treatment had no effect on fire ant recruitment (p = 0.8718). Error bars indicate + 1 SE.

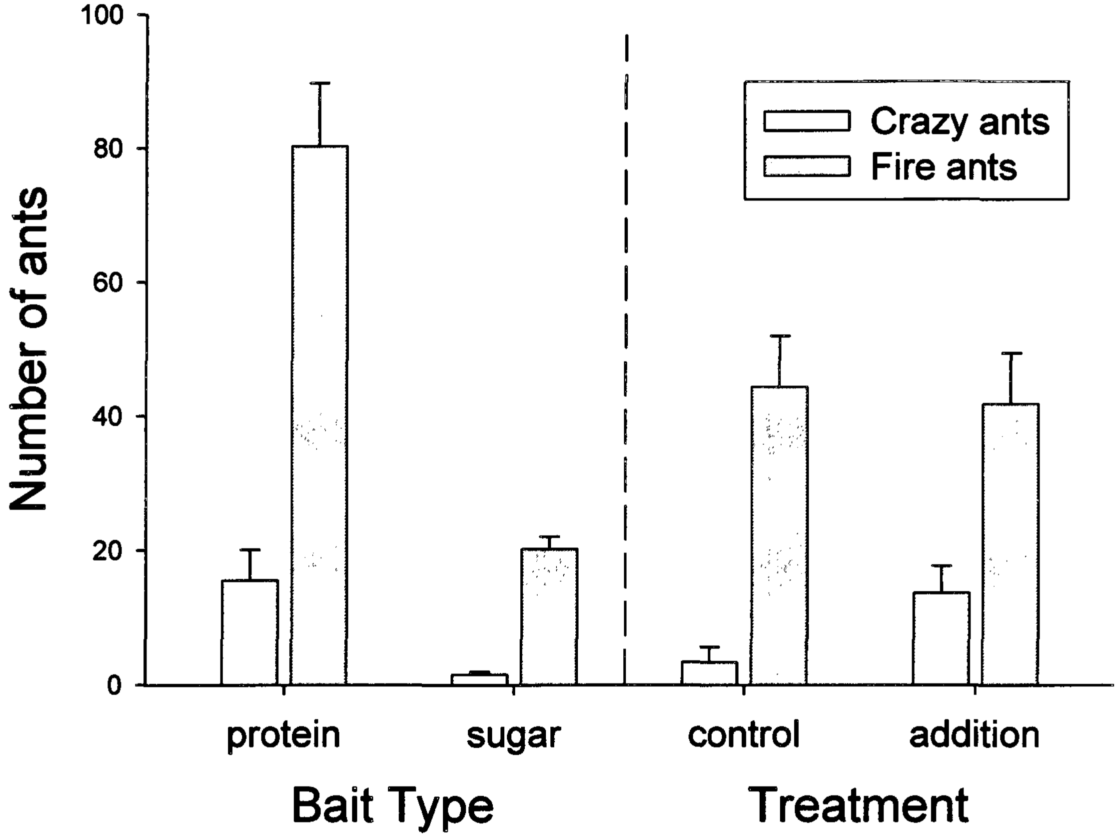

Figure 3.8. Discovery time of field baits by bait type and species. Sugar baits were discovered significantly more quickly than protein baits (p = 0.0140), and crazy ants discovered baits of both type more quickly than fire ants (p = 0.0092). Error bars indicate + 1 SE.