

Foraging Activity and Food Preferences of the Odorous House Ant (*Tapinoma sessile* Say) (Hymenoptera: Formicidae)

by

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ABSTRACT

Foraging activity and food preferences of odorous house ants (*Tapinoma sessile* (SAY)) were investigated in both the field and laboratory. Foraging activity was examined in the field from April to September 2001 by attracting *T. sessile* to feeding stations containing a 20% sucrose solution. Ant foraging activity was recorded over a twenty-four hour period along with ambient temperature to examine possible correlations with ant activity patterns. Results indicate that foraging activity may be influenced by both time and temperature. In April and May when temperatures dropped below approximately 10 °C, little or no foraging activity was observed. However, in the summer when temperatures were generally higher, foraging activity was greater during relatively cooler times of the day and night. Under laboratory conditions, *T. sessile* was attracted to feeding stations and foraged throughout the day and night at a constant temperature of approximately 25°C. Evaluations of seasonal food preferences using carbohydrate, protein and lipid samples were also conducted throughout the spring and summer. Results indicated no seasonal shifts in food preference in foraging ants; *T. sessile* consistently preferred sugar and protein rewards over lipids.

Macronutrient choice assays were performed on *T. sessile* to evaluate specific food preferences. Several different carbohydrates, proteins, lipids and salts were tested in both liquid and gel formulation. Results indicated significantly greater consumption of sucrose solution at a concentration of approximately 20% compared with other sugars tested (fructose, glucose, trehalose and maltose). In addition, strong feeding responses were observed to both casein hydrolysate and lactalbumin hydrolysate at a 5% concentration. The addition of NaCl to 15% sucrose gel samples also enhanced feeding responses. Lipids were generally ignored by *T. sessile* and in most cases decreased consumption of the sample. Various amino acids did not enhance feeding responses and were similar to water.

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DEDICATION

I dedicate this thesis to my parents. Their unconditional love and support has made this journey worth while.

I also dedicate this thesis to Antoine Latapie. Whose steadfast support and encouragement kept me motivated and held me up, during times when I could not see the light at the end of the tunnel.

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CHAPTER 1. INTRODUCTION

The odorous house ant, *Tapinoma sessile* Say, is one of only nineteen North American species in the subfamily Dolichoderinae (Borrer et al., 1989). It is commonly found throughout the United States as well as in regions of Canada and Mexico (Hedges, 1998). It was first described by Thomas Say in 1836 and by the early 1920's had established a reputation for being a major household and structural pest (Smith, 1928). The odorous house ant is currently viewed as a major pest species along the West coast of the United States, (California, Washington, and Oregon), in parts of the mid-south including Tennessee, Arkansas and Mississippi (Hedges, 1998), as well as areas along the East coast (Virginia and Maryland) (Heinsohn, personal communication, 2001; Rummel, personal communication, 2001).

The recognition of odorous house ants as a common house-infesting pest has increased the need for more effective means of control. Traditional methods of control for odorous house ants have utilized the application of insecticide sprays in and around the structures where ants have been observed. However, this approach has been largely ineffective and can exacerbate the pest problem. A more effective approach would be to use baits, a technique that can provide several advantages. One advantage is that colonies do not have to be located for control to be effective. Second, the amount of active ingredient placed into the environment can be reduced. Unfortunately, effective baits for odorous house ants are not currently available. Relatively little is known about either the foraging behavior of the ants or their feeding habits, other than a few general references to honey dew or sweet foods (Smith, 1965). A better understanding of odorous house ant food preferences and foraging behavior is needed before effective baits can be developed.

The overall object of this study was to examine the feeding preferences of the odorous house ant as a basis for bait development. The specific objectives were to:

- 1) Document foraging activity of *T. sessile* on both a diurnal and seasonal basis.
- 2) Identify macronutrient preferences of *T. sessile* with respect to season.
- 3) Identify specific nutrient preferences of *T. sessile*.

CHAPTER 2. LITERATURE REVIEW

2.1 Biology of *Tapinoma sessile*

Odoriferous house ants (*Tapinoma sessile* (Say)) are similar to other small, dark colored ant species such as the Argentine ant (*Iridomyrmex humile* (Mayr)) (formerly known as *Iridomyrmex humilis*), and the crazy ant (*Paratrechina longicornis* (Latreille)). However, the odoriferous house ant can be identified by the presence of a single node on the petiole that is hidden from view by the abdomen. The workers are monomorphic, approximately 3mm in length, and solid brown to black in color. These ants get their common name “odoriferous house ants” from the rotten coconut-like odor they emit when alarmed or crushed. The odor is caused by the release of butyric acid which is sequestered in the ant’s gaster (Thompson, 1990). This coconut-like odor is commonly used to identify these ants in the field.

Odoriferous house ant colonies are polygynous, and can number in the tens of thousands of workers. The release of male and female reproductives (swarmers) occurs during the summer months between May and July. Males emerge a few days before alate females (unmated reproductives) and it is believed that mating takes place both inside and outside the nest (Smith, 1928). Queens that mate inside the parent colony will typically stay and begin laying eggs, thus contributing to the population of the parent colony. Incipient colonies are started by potential queens that mate outside the nest. The males die soon after mating and the newly fertilized queens seek out a nest site to begin a new colony. Once a new nest has been initiated, queens lay eggs throughout the summer and fall months until November. At this time, brood production stops and the ants overwinter as workers, dealate females (queens), and partly grown larvae (Smith, 1928). In early March, workers emerge from their overwintering state and begin to forage. The queens resume egg laying in April and continue until November (Smith, 1928). Developing larvae that were present in the nest at the beginning of the winter months remain dormant until spring when they continue development, emerging as adult workers in April (Harada, 1990). The developmental period from egg to adult in spring, summer and fall months is approximately 6-7 weeks (Smith, 1928). The average time for worker

development is 7 weeks, which suggests that four to five generations of workers are produced per year.

Large colonies numbering in the thousands of workers may eventually split to form separate or satellite colonies. The splitting of a large parent colony is called budding. Budding occurs when several hundred members leave the parent colony and relocate to a new nest site, forming a satellite colony. However, ants in the satellite nest remain in contact with the parent colony by foraging trails. These trails provide for the exchange of workers, food and brood.

Odorous house ants are opportunistic nesters and will exploit many different habitats. In the field, ants build transient, shallow nests in soil, logs, stumps, under stones, leaves, boards, plastic outdoor tarps and other debris. In structures, they can build nests in wall voids, around hot-water pipes and heaters, behind paneling or beneath the floor or carpets (Hedges, 1998). They have also been found nesting in beehives and mailboxes. These ants are extremely restless and tend to relocate approximately every 25 days (Thompson, 1990). Odorous house ants can tolerate a wide range of habitats ranging from sandy beaches, pastures, and wooded areas (Hedges, 1998) to bogs, swamps and other areas of high humidity (Harada, 1990). These ants have also been found nesting from sea level to altitudes over 10,000 feet (Smith, 1928).

It has been well documented that odorous house ants will exploit many different types of nesting substrates and materials (Thompson, 1990). However, Meissner and Silverman (2001) showed that both argentine ants and odorous house ants avoid aromatic cedar mulch as nesting material. When given a choice between fresh aromatic cedar and other mulches (cypress, hardwood, pine bark and pine straw), both species of ants always colonized the non-cedar mulch. When aromatic cedar mulch was the only choice, the ants did not colonize it.

Though many accounts have been reported on the nesting behavior of odorous house ants, relatively little is known about their feeding habits. Odorous house ants will

readily seek out honeydew and will feed on dead insects (Smith, 1928). Honeydew is collected by workers that regularly tend honeydew-excreting insects, such as aphids, scale insects, mealybugs and membracids (Smith, 1965). Workers have also been observed visiting floral and extrafloral nectaries of plants (Smith, 1965). In addition to foraging on honeydew and nectar, Clark and Blom (1991) observed *T. sessile* foraging on a dead montane vole, *Microtus montanus* (Peale) in mid-April of 1987. This observation may reflect a need for protein in the spring when queens are returning to egg laying and brood rearing after an overwintering period of several months.

Odorously house ants are voracious feeders. They have been observed to feed for as much as three to five minutes before they are satisfied (Smith, 1928). While feeding, the gaster gradually enlarges until the chitinous segments are clearly separated by the intersegmental membranes causing the gaster to appear striped. The stretching of the abdomen allows odorous house ants to carry a relatively large amount of food back to the nest and share it with other colony members.

2.2 Pest Status and Control Strategies

Odorously house ants are among many house-infesting ant species that have plagued various regions of the United States. Because they are recognized as a common house-infesting pest, understanding their foraging behavior and food preferences may prove useful for developing more effective means of control. According to Heinsohn, regional entomologist of Western Pest Services in Northern Virginia, odorous house ants are their number one “call-back ant pest” (Heinsohn, personal communication, 2001). They are also the number one “call-back” pest in the Richmond, Virginia area, as well as in other areas of the state according to Western Pest Services (Rummel, personal communication, 2001).

The traditional method of control for odorous house ants includes the application of a spray formulation insecticide in and around the structures where ants have been observed. However, this method may exacerbate the problem by causing a colony to split into multiple units, each of which can become a separate or satellite colony. The

insecticide spray may also disrupt a colony causing them to relocate. A more effective approach to controlling odorous house ants would be the use of baits.

Ant baits generally consist of an attractive food source that is combined with a toxicant. The most effective ant baits have a slow acting toxicant that allows the ants to pick up the bait and bring it back to the nest where they can share it with other members of the colony through trophallaxis. This method of control provides several advantages. One advantage is that colonies do not have to be located for control to be effective. Another advantage is a reduction in the amount of insecticide placed into the environment. Baits also reduce the risk of human and animal exposure to pesticides and target specific insect pests. The use of spray applications on the other hand, are less specific and may kill non-target organisms, including beneficial insects. Spraying ants also prevents them from returning to the nest and passing the insecticide material to other members of the colony (Barbani et al., 2001). The use of chemical sprays may contaminate baits making them repellent or disrupt a colony causing it to relocate or split into multiple units. In addition to baiting, an effective ant management program should also include good sanitation and exclusion practices to prevent ants from coming indoors (Hedges, 1998).

2.3 Foraging Activity

2.3.1 Foraging strategies

When investigating foraging behavior, it is important to keep in mind that behavioral patterns in insects are generally stereotyped and incapable of much change (Hsiao, 1985). These behavior patterns are often rigidly programmed as fixed action patterns (FAPs). Therefore, each species is genetically predisposed to a specific behavior pattern for securing food (Hsiao, 1985).

Foraging behavior for most animals may involve complex strategies to help locate resources in the environment. In ants, it is a collective process composed of both the activities of individuals, as well as the activities of the integrated group (Traniello, 1989). A colony must harvest food efficiently and minimize resource competition during individual and colony-wide foraging (Traniello, 1989).

To maximize their success while foraging, ants often use different types of behavior that can be correlated to colony size (Beckers et al. 1989). Studies conducted on 98 different ant species revealed that smaller colonies tend to rely on individual foragers that do not communicate their discoveries to the rest of the colony. However, larger colonies rely on chemical communication between individuals of the same colony and employ mass recruitment systems. These systems allow a single forager to communicate foraging success to a larger number of individuals (Beckers et al. 1989).

Foraging systems and ant activity are also subject to several limiting factors. These factors may include predation, humidity, time of day and even season. However, the most important limiting factor is probably temperature. Klotz (1984) observed temperature effects on both ant activity and movement. He recorded a reduction in foraging activity in *Formica subsericea* at cooler temperatures (below 12 °C) and a reduction in the *speed* of movements at temperatures between 14 and 18°C.

Trail experiments performed on carpenter ants (*C. pennsylvanicus*) by Klotz et al. (2000) indicated that low temperatures were also found to be an important constraint that was magnified in ants traveling through vegetation. Klotz et al. found that carpenter ants traveled significantly faster on structural guidelines than on the ground through vegetation. As ambient temperatures decreased, ant travel through vegetation slowed significantly when compared to ants traveling on structural guidelines. Structural guidelines used in testing simulated preexisting structures in their environment (edges and grooves). The ants essentially trailed in a straight line, even in the absence of structural guidelines.

Foraging strategies used by ants to find and exploit food sources may also differ from species to species. For example, black carpenter ants (*Camponotus pennsylvanicus* (DeGeer)), are mainly active at night while other species are active during the day (Cannon, 1998). This strategy reduces the problem of competition between neighboring ants and may help avoid predation or paritization (Hölldobler and Wilson, 1990).

Foraging activity patterns in the southern fire ant (*Solenopsis xyloni*), were investigated by Hooper and Rust (1997). A granular anchovy diet was placed into vials that were then positioned near 4 colonies of *S. xyloni*. The ants were allowed to forage for 24 hours. Results indicated a significant increase in foraging at sunset with peak activity occurring between 2 and 7 hours later. Foraging was observed to commence approximately 4 hours before sunset and terminate approximately 1 hour before sunrise. *S. xyloni* foraged at night from May to November with most of the foraging activity occurring in June, August and October. In addition, Hooper and Rust observed no foraging activity in *S. xyloni* from December through April.

2.3.2 Foraging Activity of *Tapinoma sessile*

T. sessile is known to be active both day and night, relying predominately on visual cues and odor trails to locate resources. They also have a tendency to follow structural features in the environment while foraging. Klotz and Reid (1992) examined the topographic orientation of both *T. sessile* and *Camponotus pennsylvanicus* as they foraged from their nest to a food source, documenting their responses to a series of cues while utilizing structural guidelines. Klotz and Reid referred to this particular behavior as structural guideline orientation and classified four types of structural guidelines (bilateral-elevated, bilateral-depressed, unilateral-elevated and unilateral-depressed) that were used by the ants as they oriented along a given surface (**Figure 1**). The guidelines simulated preexisting structures and provided a means by which guideline orientation could be compared with the use of odor trails. Their results demonstrated that gravity is an important cue in orientation especially when ants are foraging for honeydew in trees. On a vertical plane, edge orientation was more prominent. However, when foraging on a horizontal edge, ants tended to leave the edge to take a shorter route to the food source.

Klotz and Reid (1992) also examined the role of visual cues in the establishment of foraging trails. These tests were done in both daylight and darkness. When ants were deprived of visual cues, they rarely deviated from the unilateral edge and relied extensively on tactile stimuli when orienting to the food source. However, when ants were provided with visual cues, they tended to deviate from the edge more readily. Both *T. sessile* and *C. pennsylvanicus* relied heavily on visual cues for direction and distance information. For *T. sessile*, the odor trail was secondary in importance to visual cues.

Structural Guide Lines

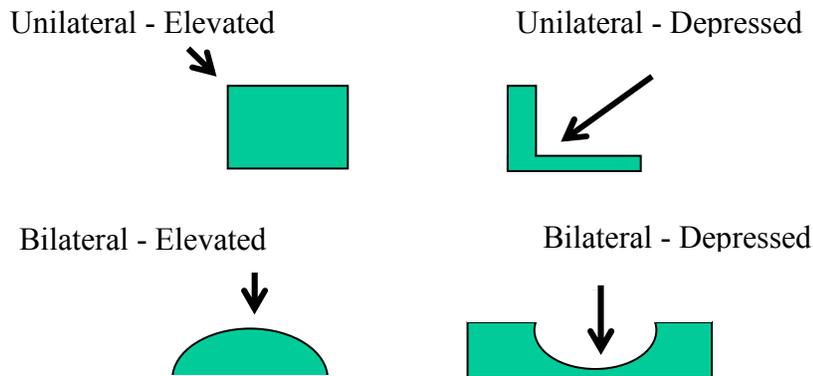


Figure 1: Types of structural guidelines used by odorous house ants for orientation (Klotz and Reid, 1992)

Klotz et al. (2000) conducted additional studies on structural guideline orientation using *T. sessile* and other species of ants. Indoor colonies of *T. sessile* that were first baited to initiate foraging trails showed a strong tendency to use structural guidelines (between 91.8 – 59.9% of the time). The ants were observed to exit a hole in a wall, and follow molding and grooves in the wood paneling to reach a food source on the kitchen counter. They were also found to emerge from beneath cabinets and follow the edges to reach honey on top of a refrigerator.

Foraging activity of *T. sessile* was also investigated by Bernstein (1979). Bernstein considered temperature when examining the foraging activity of *T. sessile* and other species of the Mojave Desert and Great Basin. In this study, he showed that as elevation and latitude increased, foraging temperature ranges become broader and the overlap in foraging time between ant species became greater. The range of foraging temperature for *T. sessile*, at an elevation of 1500m was recorded to be between 6 and 35° C. This range was smaller than that reported by Hunt (1974) for *T. antarcticum*, (16-50 °C at an elevation of 1000m).

2.4 Food Preference

2.4.1. Food Preference of *Tapinoma sessile*

Currently, little is known about specific food preferences of *T. sessile*, except that they consist primarily of sweet foods, such as honeydew, and dead insects. Past reports on food preferences consist mainly of anecdotal and general observations of feeding on complex foods such as meat grease, and processed foods such as jams and jellies. Wang and Brook (1972) conducted feeding studies on odorous house ants by offering such foods as: apricot and blackberry jam, apple butter, meat grease, cottonseed oil, orange juice, and syrup. Their results indicated that odorous house ants were mostly attracted to syrup, apricot jam and blackberry jam. Unfortunately, these results do not indicate specific nutrient preferences among different sugars, nor do they reveal preferences among varying concentrations of food types.

Smith (1928), conducted biological studies on the odorous house ant and found that under normal conditions they are largely honeydew loving insects. Smith also reported accounts of odorous house ants entering homes and cutting through paraffin in order to reach jelly and preserves in containers. They were noted to infest honey, sugar, pies, custards and marmalades. In addition to the sweet foods, odorous house ants were found infesting uncooked beef, fish, boiled and mashed potatoes, cheese, milk and ripe fruits.

Smith (1928) found that odorous house ants may also forage on unusual food sources to survive. In 1969, Brown noted that several odorous house ants became trapped inside one of his Kodachrome® slide boxes. To survive the ants apparently fed on the gelatin-coated film base of the Kodachrome slides. The ants used their mandibles to remove the dark dyed gelatin emulsion from the transparent film base. Feeding sites were seen as small white spots at the edge of the film area by the naked eye. However, magnification of these feeding sites revealed numerous gouges of odorous house ant's mandibular teeth. In addition, all feeding sites were restricted to the extreme edge of the slide and no sites extended further than 1.0mm from the edge of the film.

2.4.2. Food preference of other ants

Baker et al., (1985) performed bait-preference tests on argentine ants *Linepithema humile* (Mayr). Foods such as sucrose water, tuna meal, and corn meal were tested on the ants. Results revealed that argentine ants preferred liquid, sugary foods to all other types of food; in particular 25% sucrose solution and 25% honey solution. When proteinaceous substances were added to a 25% sucrose solution, feeding was often reduced. For example, a reduction in feeding on sucrose solutions containing as little as 0.3% casein hydrolysate was observed. Egg white on the other hand, was the only substance tested that did not lower feeding levels, and led to a significant increase in consumption.

Silverman and Roulston (2001) compared the feeding responses of the Argentine Ant, *L. humile* (Mayr), when offered liquid and gel formulations of sucrose. Their results indicated a greater number of foragers visited the gel formulation but substantially more liquid was consumed. The workers could stand on the gel and therefore more foragers could feed on it simultaneously. However, ants were less efficient at extracting sucrose from the gel than from the liquid. When fipronil was added to the different formulations, a greater proportion of the nest died after foragers fed from the liquid than from the gel baits.

Hooper and Rust (1997) investigated food preferences of the southern fire ant (*Solenopsis xyloni*). The investigators incorporated a combination of readily available food products into a bait mixture and conducted choice tests to determine the most attractive diet. The original diet (described by Keller et al., 1989) contained beef hash as the primary source of protein. Hooper and Rust replaced this protein source with tuna, anchovy, sardine or mealworms. The diet was then baked or freeze-dried to formulate granules of varying particle size. Their results indicated that diets containing anchovy were preferred over the other diet combinations. Unfortunately, it is unclear as to whether the ants were actively consuming the diet or just moving the material around.

Food preference assays were conducted by Tripp et al., (2000) to better evaluate seasonal food preferences in the black carpenter ant (*C. pennsylvanicus*). In this study, foraging ants were provided a choice between protein (i.e. diced mealworms) and carbohydrates (i.e. clover honey) during June and August. The results indicated a shift in preference from proteins in early June, to carbohydrates in late August. However, it is unclear from the experimental design, exactly what kinds of macronutrients were being consumed or removed. Diced mealworms contain a variety of macronutrients other than proteins, sugars and lipids, and honey contains several different types of simple sugars.

Cannon (1998) also conducted feeding studies on *C. pennsylvanicus* in an attempt to better define their feeding preferences. Specific lipids, proteins and carbohydrates were tested on the ants in varying concentrations. The results indicated that foragers demonstrate a preference for sucrose, fructose and glucose. These are sugars commonly found in honeydew. Honeydew, is primarily a carbohydrate-rich food source, but can also contain other nutrients such as amino acids, amides, lipids, sugar alcohols, organic acids, sterols, salts, minerals and B-vitamins (Fell and Morse, 1977). Cannon (1998) also investigated the consumption rates of simple sugars at different concentrations. Sucrose and fructose produced the highest overall consumption at a concentration of 20%. At greater concentrations sugar consumption leveled or declined. Protein hydrolysates appeared to be favored over other types of proteins such as albumin. Casein hydrolysate, in particular, was extremely effective at stimulating feeding in ants both in the laboratory and in the field. Varieties of animal and plant-derived lipids were also tested for consumption with egg yolk being the most attractive and lard being the least acceptable. Overall, it was shown that *C. pennsylvanicus* collected mainly carbohydrate and nitrogen compounds. Pure lipids of both plant and animal origin were not attractive to the ants.

Carpenter ants have also been the subject of gustatory preference tests using urea and sugars (Shetty, 1981). In general, the consumption of urea by *C. compressus* was greater than that of sucrose. Active feeding on urea solutions (0.01 – 10M) began at 0.1M and increased rapidly with increasing concentrations, finally leveling at concentrations over 0.5M. In addition, urea was preferred over sucrose, maltose,

fructose, glucose and galactose when tested at similar concentrations. Urea solutions were consumed at 0.1M whereas sucrose solutions were not consumed until a concentration reached 0.3M or higher.

In addition to many macronutrient compounds, amino acids have been the subject of food preference research in some ant species. Ricks and Bradleigh (1970) investigated the acceptability of various amino acids, sugars, vitamins and extracts of adult arthropods to two species of *Solenopsis*. Various insects extracts, as well as whole and crushed arthropods, were found to be readily accepted by the ants. The consumption of amino acids was equal to or less than water. However, leucine was the exception, as it was more attractive than water. In addition, both species of ants showed similar preferences for melezitose, sucrose and glucose but rejected xylose, ribose, mannose, arabinose, and galactose. Vitamins were also tested and folic acid, B₁₂, and inositol were preferred over water. None of the B vitamins tested were more attractive than the preferred sugars or amino acids.

Lanza et al. (1993) compared *S. invicta* and *S. geminata* feeding preferences when offered amino acids and sugar components found in extrafloral nectars. Nectar mimics were prepared that varied in either amino acid or sugar concentrations. The fire ants were then fed pre- and postdefoliation nectars of *Impatiens sultani*, which varied in amino acid content. Both ant species were also fed from artificial nectaries containing mimics of nectar from *Passiflora ambigua*, *P. talamancensis*, and *P. quadrangularis* which varied in sugar content. The results indicated that *S. geminata* workers preferred the postdefoliation nectar mimic (rich in amino acid content) over the predefoliation mimic (which contains a relatively lower amino acid content). *S. invicta* however, did not discriminate between the two nectar mimics. In sugar preference tests, both ant species preferred the nectar of *P. ambigua* over *P. talamancensis* and *P. quadrangularis*. It is interesting to note, that the nectar of *P. ambigua*, preferred by both species, contained the lowest total sugar concentration and the lowest caloric content. The preference exhibited by *P. ambigua* may indicate that these ants do not necessarily maximize their sugar

energy intake when foraging, contrasting with the expectations of optimal foraging theory (Pyke, 1984).

The consumption of specific amino acids by *Leptothorax* and *Monomorium* species was investigated by Lanza and Krauss (1984). The two ant species were offered choices among various amino acid samples commonly found in floral and extrafloral nectars. Both ant species preferred solutions prepared with a single amino acid (alanine, arginine, serine, cysteine, methionine or aspartic acid) compared with control samples of sugar solutions. *Monomorium* preferred control samples over tyrosine solutions whereas, *Leptothorax* preferred control solutions over histidine solutions. In addition, *Leptothorax* did not discriminate between the control and tyrosine solutions. *Monomorium* did not discriminate between the control and histidine solutions.

2.5 Toxicity of Insecticides in Ant Baits

Few contact toxicity studies have been performed on the odorous house ant. Chong et al., (1998) exposed odorous house ant workers to varying concentrations of etofenprox and bendiocarb. They found that *T. sessile* were more susceptible to bendiocarb ($LC_{50} = 0.014\mu\text{g}/\text{cm}^2$) than to etofenprox ($LC_{50} = 0.450\mu\text{m}/\text{cm}^2$). However, in similar studies on other common household ants (*M. pharaonis*, *P. longicornis*, and *H. bituberculata*), Chong et. al. (1998) found that *T. sessile* was more tolerant to bendiocarb than *M. pharaonis*, *P. longicornis*, and *H. bituberculata*. The author suggested that the superior killing efficiency of bendiocarb was probably due to its lower repellency and detoxification rate by infected worker ants.

Klotz et al. (1997) tested the toxicity of boric acid-sucrose water bait on *Solenopsis invicta* (red imported fire ant). Liquids from honeydew, plant sap and nectars were found to comprise a major portion of the ant's diet. Boric acid was found to be effective in ant baits due to its non-repellent and slow-acting nature at low concentrations. In addition, they found that exposing fire ants to 0.25, 0.5, 0.75 and 1% boric acid sucrose solution baits effectively reduced colony size by more than 90% after 6

weeks. It was also found that high doses of boric acid (5%) increase the likelihood of ant avoidance. High doses would also kill foragers more quickly and thus reduce trophallaxis. Similar findings were reported with the use of liquid boric acid baits for control of pharaoh ants (Klotz et al. 1997).

Costa and Rust (1999) exposed colonies of argentine ants (*Linepithema humile*) to potted oleander plants containing soil that had been treated with an insecticide. The insecticide (either diazinon (5G) or fipronil) was applied as a broadcast spray or incorporated into the soil as a soil-mixed treatment. Mortality and foraging rates of argentine ants were observed and recorded for several weeks after the treatments. The authors observed a reduction in foraging rates that was similar in all treatments. However, mortality rates varied among the different insecticides and different application methods. Broadcast treatments of fipronil and diazinon took longer to kill workers and queens than the soil-mix treatments. Mortality rates were the greatest in soil-mixed treatments of fipronil, and only the fipronil treatments killed the queens. In addition, only fipronil soil-mix treatments prevented ants from establishing colonies in the potted soil.

An evaluation of several ant baits and spray treatments for the control of the black carpenter ant was conducted by Tripp et al. (2000). In a bait acceptance study, three bait granules were evaluated: Maxforce™, Niban™, and Baygon™. One hundred bait granules from each of the three baits were offered to several ant colonies. After thirty minutes of testing, the baits were removed and the remaining bait granules were counted. Results showed that Maxforce™ bait granules were preferred over Niban™ and Baygon™ in the spring, but there was no preference in the fall.

Tripp et al. (2000) evaluated the residual toxicity of Dursban™ 50W and Tempo™ 20WP as it relates to the control of carpenter ants over a period of several months. Their study showed that both Dursban™ 50W and Tempo™ 20WP exhibited a lower insecticidal activity when applied to paneling exposed to direct sunlight than in the shade.

Catanguì et al., (1996) investigated the abundance and diversity of ants on different rangelands treated with diflubenzuron. The authors concluded that the abundance of ants was not significantly reduced by aerial applications of Dimilin™ 2F, Dimilin™ 25W, or Sevin™ 4-oil. Diversity declined temporarily on days 13-19 following treatments with Dimilin™ 25W. However, densities immediately recovered a week later with no further declines for up to 356 days. These authors suggested that the observed reduction in population may have been due to the natural decline in the ant colony in preparation for winter.

Gibson and Scott (1989) tested fourteen insecticides (3 carbamates, 7 organophosphates, and 4 pyrethroids) on two species of carpenter ants (*C. novaeboracensis* and *C. pennsylvanicus*) using residual bioassay tests. They found that Deltamethrin™ and Diazinon™ were the most toxic to *C. novaeboracensis* and *C. pennsylvanicus*, respectively. Pyrenone and propoxur were found to be the least toxic. Gibson and Scott (1989) also suggest that in the development of an effective ant management program, fast-acting contact insecticides are the most effective when the nest is located and accessible to treatment. However, if the nest cannot be located or easily treated, the preferred method of control would be the use of a slow-acting insecticide in the form of bait.

CHAPTER 3 FORAGING ACTIVITY AND SEASONAL FOOD PREFERENCES OF THE ODOROUS HOUSE ANT (*Tapinoma sessile* (SAY))

3.1 Introduction

Ants explore their environment by sending foragers out into the field in search of food for the colony. These foragers are generally the oldest individuals in the colony and the most expendable (Hölldobler and Wilson, 1990). Their task is to locate food sources and bring them back to the nest, or recruit nest mates to the resource (Hölldobler and Wilson, 1990).

Foraging may involve complex strategies to help the ants locate resources in their environment. Foraging is a collective process composed of both the activities of individuals, as well as the activities of the integrated group (Traniello, 1989). A colony must harvest food efficiently and minimize resource competition during individual and colony-wide foraging (Traniello, 1989). Foraging strategies used by ants to find and exploit food sources differ from species to species. For example, black carpenter ants (*Camponotus pennsylvanicus*) are mainly active at night, while other species are active during the day (Cannon, 1998).

To date, little is known about the foraging activity or seasonal food preferences of the odorous house ant, *Tapinoma sessile* (Say). Odorous house ants are among the many house-infesting species that have plagued various regions of the United States (Thompson, 1990). According to pest management companies (Western Pest Services and Dodson Brothers Pest Control) in 2001, the odorous house ant has become their number one call-back ant pest, as it is difficult to control once it has become established in an urban setting. Because they are recognized as a common house-infesting pest, understanding their foraging behavior and food preferences may prove useful for developing more effective means of control.

Understanding peak activity cycles are important for conducting food preference studies. Feeding studies should be conducted during times of peak foraging activity for maximum response and food consumption. Information on activity cycles and food preferences should also prove beneficial in the design of control measures using baits. Baits should be placed along foraging trails when ants are the most active to insure effective control. In this study, the daily and seasonal foraging activity cycles of the odorous house ant were examined, as well as food preferences throughout the spring and summer.

3.2 Materials and Methods

Both field and laboratory colonies were used to investigate foraging cycles and general food preferences of the odorous house ant during the spring and summer months of 2001.

3.2.1 Collecting and Maintaining Laboratory Colonies

Colonies of *Tapinoma sessile* were located around the Blacksburg area in Montgomery County, Virginia. Once the colonies were located, they were transferred into 18-quart plastic containers and brought back to the Dodson Urban Pest Management Research Laboratory at Virginia Polytechnic Institute and State University for study under controlled conditions. Ant colonies were maintained at $25^{\circ}\text{C} \pm 3^{\circ}\text{C}$. Colonies were provided with hard wood mulch as a substrate and nest material, and water filled vials fitted with a cotton wick for continuous access to water. A spray bottle was used daily to mist water into the containers to keep the environment moist. Mineral oil was used to coat the inner surface of the containers to prevent escape. All colonies were maintained on a diet of a 20% sugar solution and a supply of dead insects. Colonies were allowed to acclimate to the laboratory conditions for approximately 3 days and starved for at least 2 days prior to testing.

3.2.2 Foraging Activity Tests

Initial laboratory tests utilized four colonies of odorous house ants. Feeding stations consisted of 20% sucrose water placed into polystyrene weighing dishes (Fisherbrand®), approximately 4cm² in size (**Figure 2**). The weighing dishes (feeding station) were placed inside each of the 18-quart plastic containers. The number of foraging ants feeding at the station was recorded every hour for 24 hours. Temperature was maintained at approximately 25 °C.

3.2.3 Field Sites

To study foraging cycles under natural conditions, field colonies were located and marked with construction flags. Because odorous house ants are sensitive to mechanical disruptions and tend to relocate approximately every 25 days (Thompson, 1990), new colonies of *T. sessile* were located every month for study. Colonies were baited to a feeding station that consisted of 20% sucrose solution in a polystyrene weighing dish (**Figure 2**) for at least 2 hours before counts were made. Direct observations were made of the number of ants at the feeding stations every hour for 24 hours in April, May and June. Temperature data were collected at each site using a microprocessor thermometer model HH23 (Omega Engineering, Inc.).

Foraging activity in June, July and September was recorded using time-lapse photography and a digital clock equipped with a temperature gauge. The camera used in this setup was a Canon™ AE-1 Program, equipped with the Canon Interval Timer TM-1 Quartz set at 30-minute intervals. A macro lens (Canon™ Lens FD 100mm) was coupled with an O-ring flash (Olympus™ T 10 Ring Flash) and focused directly at the feeding station and digital clock (**Figure 2**). The camera was fixed onto a tripod and positioned a few feet above the feeding station. The camera was also connected to a 120V power source via an extension cord and an umbrella was used to protect the camera from direct sunlight (**Figure3**).



Figure 2: Foraging activity of *Tapinoma sessile* was recorded in June, July and September using time lapse photography. Odorous house ants are shown foraging at the rim of the polystyrene weighing dish (approximately 4cm²). The dish (feeding station) contains 20% sucrose solution. The number of foraging ants feeding at the dish was recorded every hour for 24 hours. To the right of the feeding station is a digital clock used to capture time and temperature (°C).



Figure 3: Foraging activity of *Tapinoma sessile* was recorded throughout the summer of 2001. Time-lapse photography was used to capture foraging activity at a feeding station containing 20% sucrose solution. The camera used in this setup was a Canon™ AE-1 Program, equipped with the Canon Interval Timer TM-1 Quartz set at 30 minute intervals. A macro lens (Canon™ Lens FD 100mm) was coupled with an O-ring flash (Olympus™ T 10 Ring Flash) and focused directly at a feeding station.

3.2.4 Seasonal Food Preference Tests

Odoriferous house ant macronutrient preference tests were conducted every month from May to August using laboratory colonies. Each month new colonies were located in the field and brought back to the laboratory where they were established as previously described (**Section 3.2.1**). The colonies were allowed to acclimate to the laboratory for approximately 3 days before testing was initiated. The colonies were starved for at least 2 days prior to testing; food was withheld and only water was provided. The initial food preference tests consisted of offering the ants a choice among food samples consisting of carbohydrates, lipids and proteins.

Carbohydrate Samples. Preliminary tests with odoriferous house ants indicated a strong feeding response to sucrose at concentrations between 15-20%. Therefore, the carbohydrate test samples consisted of 20% sucrose (by weight) mixed with agar to provide a gel matrix. The agar gel was prepared by boiling 10ml of distilled water and slowly adding 0.24g of agar (Sigma Chemical Company) to the boiling water while stirring. In a separate beaker, 5.1g of sucrose (Sigma Chemical Company) was dissolved in 10ml of distilled water. When the agar was completely dissolved, the sucrose solution was slowly added to the agar while stirring. Aliquots of this mixture were transferred into 0.5ml centrifuge tubes (Fisher Scientific) and placed directly into an ice bath for rapid cooling. When the samples jelled, the tubes were labeled, sealed and stored in the refrigerator at approximately 4.0 °C.

Lipid Samples. Lipid samples consisted of 2.5% palmitic acid and 2.5% extra virgin olive oil formulated in an agar gel matrix. Preliminary experiments using different lipid materials have shown extra virgin olive oil and palmitic acid to be slightly attractive, or at least non-repellent to different ant species. The lipid samples were prepared in a similar manner to the carbohydrate gel samples except that they were formulated as emulsions. After the agar was dissolved in boiling water, 0.54g of palmitic acid was rinsed into the agar water mixture with 5ml of distilled water while stirring continuously. This procedure was repeated using 0.54g of extra virgin olive oil. The

emulsion was transferred into 0.5ml centrifuge tubes sitting in an ice bath to rapidly cool the mixture and maintain the lipid suspension. When the samples jelled, the tubes were labeled, sealed and stored as previously described.

Protein Samples. Casein and casein acid hydrolysate (5% by weight) (from bovine milk, Sigma Chemical Company) were used for testing feeding responses to proteins. In past experiments, casein and casein hydrolysate have shown good feeding responses with different species of ants (Cannon, 1998). The samples were prepared as previously described using 0.24g of agar in a total of 20ml of distilled water and 1.07g of either casein or casein hydrolysate. (Sigma Chemical Company).

Test Procedures. The carbohydrate, lipid, and protein food samples were used in seasonal feeding tests by offering foragers a choice among the four macronutrient foods. Sample tubes from each macronutrient group were weighed gravimetrically immediately before testing using an analytical balance XA-200DS (Fisher Scientific). The analytical balance was calibrated using standard weights; the error was less than 1%. The tubes were then placed in a random array inside containers housing odorous house ants (**Figure 4**). Control tubes consisted of either plain agar or agar containing 15% sucrose. The ants were allowed to feed at the sample tubes for up to 24 hours, after which, the tubes were removed and re-weighed (mg). Preferences were determined by measuring consumption and then converted to percent consumption to account for differences in colony size.



Figure 4: Food preferences of *Tapinoma sessile* were tested by offering the ants a choice among various macronutrient samples. Samples were formulated as either a liquid solution or gel matrix and transferred into 0.5ml plastic centrifuge tubes shown above. Tubes were placed in a random array inside a container containing a colony of *T. sessile*. Preferences were determined by measuring consumption.

3.2.5 Statistical Analysis

Field Foraging Activity (Trigonometric (Periodic)) Regression

The percentage of ants foraging for a single twenty-four hour period in the months of April, May, July and September of 2001 was analyzed to determine if there was a relationship between temperature and foraging activity. In this study, the response variable (y) is the percentage of ants foraging, while the explanatory variables are time of day or night, converted to 360 degrees (2π radians denoted by θ) and temperature °C. The purpose of this analysis was to fit a regression model to predict the mean value of y (% foraging) given θ (time of the day) and x (temperature °C of the day). This analysis is referred to as a linear-(circular+linear) association (Batschelet, 1981; Jammalamadaka et al., 2001).

A multiple regression model for this type of association has the form:

$E(Y / \Theta = \theta, X = x) = a_0 + b_0 \cos(\theta - \theta_0) + cx$, which can be rewritten as
 $E(Y / \Theta = \theta, X = x) = a_0 + a \cos \theta + b \sin \theta + cx$, where a_0 is the Y-intercept,
 $a = A \cos \theta_0$, $b = A \sin \theta_0$, $A = (a^2 + b^2)^{1/2}$ is called the amplitude, and θ_0 is called the acrophase angle (particular phase angle where $E(Y / \Theta = \theta, X = x)$ reaches its highest peak) (Batschelet, 1981).

In the latter form, it is a multiple linear regression model (linear in the regression variables $\cos\theta$, $\sin\theta$ and x) and can be fitted routinely by methods in any general statistical package (eg. SAS Institute, 1988). Fitting a trigonometric polynomial to these data is recommended so long as there are at least 6 equally-spaced time intervals (Batschelet, 1981). In this experiment, data were collected at every hour, to give a total of 24 equally spaced observations in each month; April, May, July and September of 2001.

Seasonal Food Preferences

An independent randomization, identically replicated, randomized complete block design (I,IRRCBD) (Hinkelmann and Alcorn, 1998) was chosen to analyze food preferences over time using days as blocks. Data tested in one day were analyzed as a randomized complete block design using colonies as blocks. Seasonal food preference tests were analyzed using Analysis of Variance (SAS Institute, 1990). Values of $P \leq 0.05$ were used to indicate significance. Means were separated using Fisher's Protected Least Significant Difference. Standard error generated using LSMeans corrects for variations among different treatments. LSMeans may have different variances; however in most of the analyses the variances were equal, hence equal standard error for all treatments (Hinkelmann and Kempthorne, 1994).

3.3 Results

3.3.1 Laboratory Foraging Activity

The results of initial 24-hour foraging activity studies conducted in the laboratory in August of 2000 are shown in **Figure 5**. Large numbers of foragers were attracted to the food stations almost immediately after placement of the stations. Following this initial rush of activity, foraging numbers declined and remained somewhat constant throughout the day and night. No prominent peaks of activity were observed and the general cycle of activity was consistent over the four colonies that were tested.

Odorous House Ant Foraging Activity – August 2000

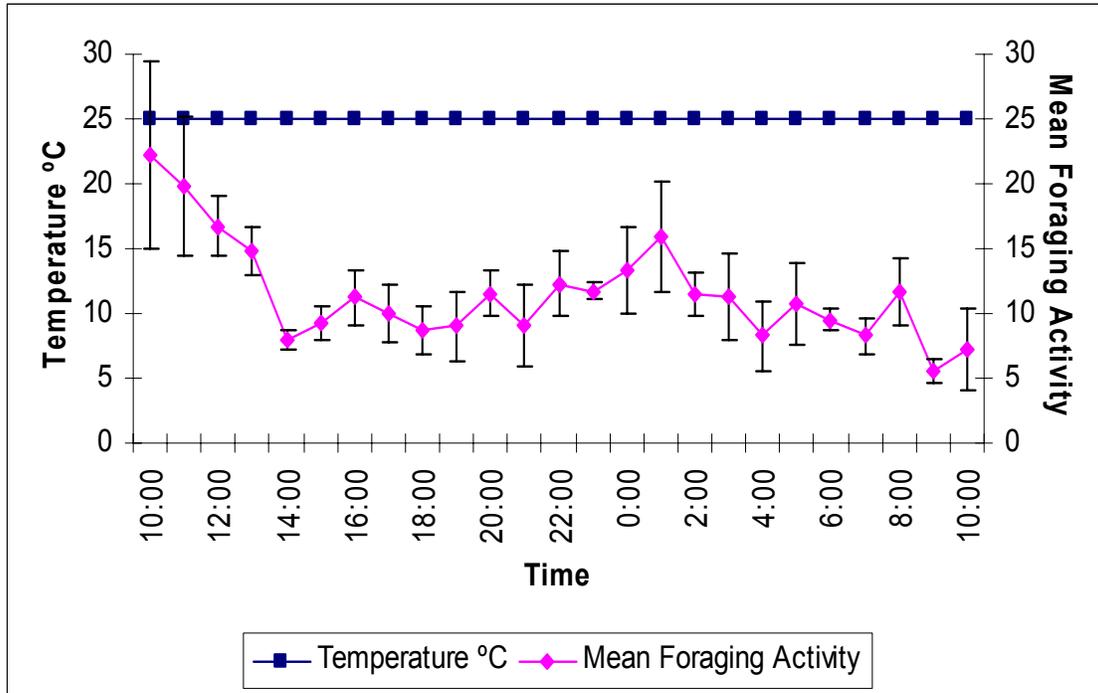
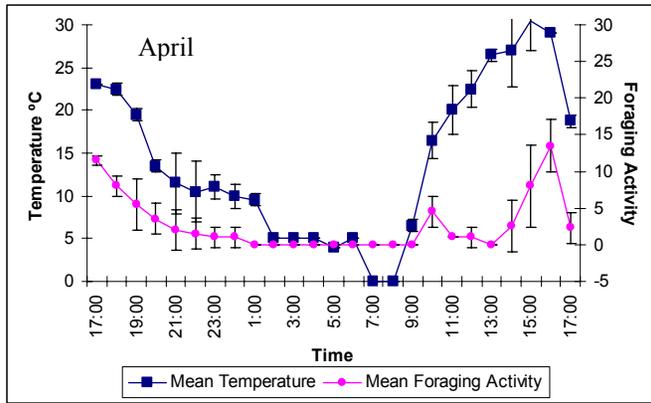


Figure 5: Mean twenty-four hour foraging activity of 4 colonies of *Tapinoma sessile* conducted in the laboratory in August 2000. Colonies were baited to a feeding station containing 20% sucrose solution. Temperature was maintained at approximately 25 °C.

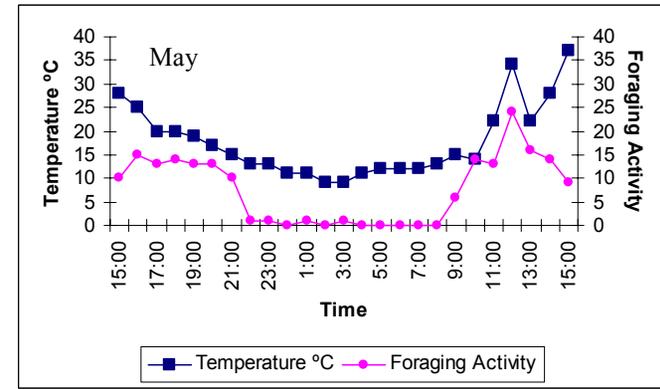
3.3.2 Field Foraging Tests

Figure 6a shows mean foraging activity and mean ambient temperatures of colonies 1 and 2 of odorous house ants located in April 2001. Field colonies of odorous house ants were relatively small in the spring and activity was shown to be restricted to times when temperatures were above approximately 10 °C. Activity declined in April with decreasing temperatures and no foraging activity was observed between 1:00 to approximately 10:00. At approximately 13:00, foraging activity seemed to slowly increase as temperatures began to rise. A peak of activity was observed at approximately 16:00 when temperatures reached approximately 30 °C. A sharp drop in foraging followed this peak of activity with decreasing temperatures. Data collected in April were analyzed using trigonometric regression. Foraging activity in April was shown to be dependent on time and temperature. When temperatures dropped, activity also declined (**Figure 7**).

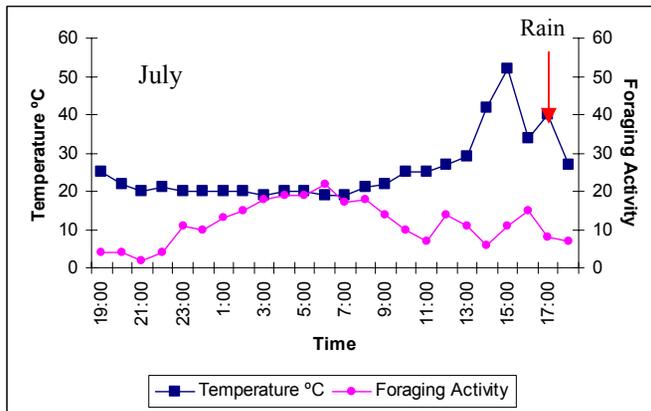
In May, two different colonies of odorous house ants were followed, colonies 3 and 4. During the experiment, small honey ants took over the food source for colony 3 approximately 6 hours after the start of the study. The small honey ants utilized strong foraging trails throughout the night and did not seem to be as affected by the drop in temperatures as did the odorous house ants. Foraging activity in colony 4 however, was active until 22:00 (**Figure 6b**). As temperatures fell below approximately 12 °C, foraging declined to the point of inactivity. When temperatures gradually increased around 9:00, foraging activity in colony 3 resumed. Results from the trigonometric regression analysis of colony 4 indicate foraging pattern similar to what was observed in April (**Figure 7**). Predicted values generated by the model closely match the observed data. As temperatures fell below approximately 12 °C, foraging declined to the point of inactivity.



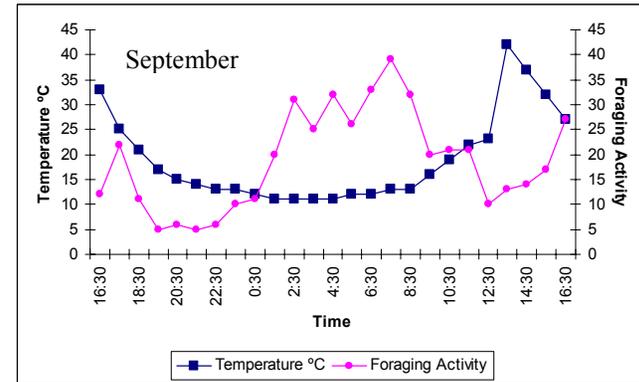
a)



b)



c)



d)

Figure 6: Twenty-hour foraging activity of *Tapinoma sessile*. Foraging activity was recorded on a randomly chosen day at the end of each month on (a) April 26, 2001, (b) May 29, 2001, (c) July 30, 2001, and (d) September 18, 2001. Field colonies were located and baited to feeding stations containing 20% sucrose solution. The number of foragers feeding at the food station was recorded every hour for twenty-four hours. Legend: temperature °C in blue with square points and the number of foraging ants counted at food stations is pink with round points.

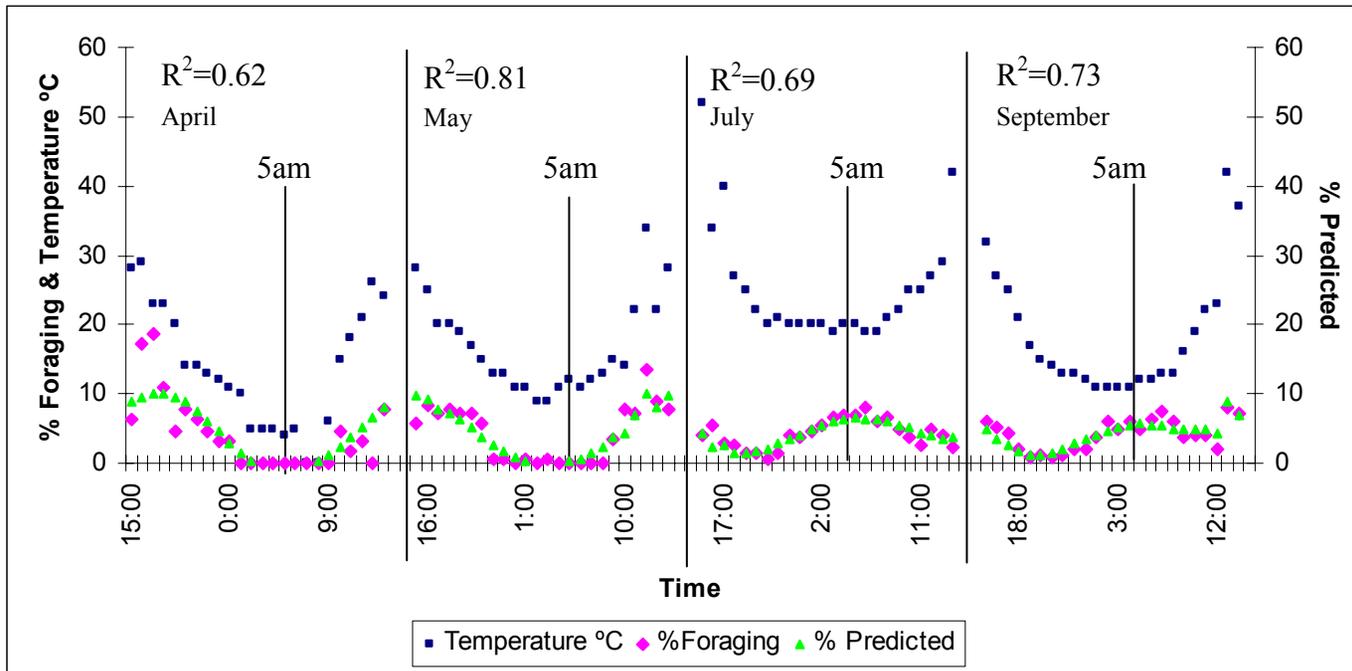


Figure 7: Trigonometric regression was used to analyze foraging activity of *Tapinoma sessile*. Colonies were observed in the field on a randomly chosen day at the end of each month in April, May, July, and September 2001. *T. sessile* were baited to a feeding station containing 20% sucrose solution and activity was determined by the number of ants feeding at the stations every hour for twenty-four hours. In April and May activity declined with decreasing temperatures and was shown to be restricted to times when temperatures were above approximately 10 °C. In July and September there is an inverse relationship between temperature °C and activity. Activity in the mid to late summer increased during times when temperatures were generally cooler.

In June, two colonies of odorous house ants were followed (colony 5 and 6). A similar case of forager displacement by competing species occurred with colony 5. Approximately ten hours after the onset of the study, small honey ants took over the food station and successfully displaced the odorous house ants from the food source. This displacement of the odorous house ants made it impossible to collect accurate foraging data. Colony 6, on the other hand, was very active throughout the day and night but generally did not respond to the feeding station. This colony was observed collecting and moving brood to several different locations throughout the night. Odorous house ants from colony 6 generally ignored the feeding station while in the process of relocating from one site to another. As a result of their non-responsiveness to the feeding station, acrobat ants took over the food source. The lack of foraging activity by colony 6 and the interference caused by competing ant species in colony 5 prevented the collection of accurate foraging activity data for the month of June. Therefore, a colony of odorous house ants was collected and brought to the laboratory where foraging activity could be observed under controlled conditions.

The results of the June laboratory tests were similar to the preliminary laboratory results recorded in August 2000 (**Figure 8**). Foraging ants immediately rushed the food source, after which, foraging dropped and relatively low levels of activity were maintained throughout the day and night. Aside from the initial rush of foragers to the food station, no obvious peaks of activity were identified during this test.

Odorous House Ant Foraging Activity – June 2001

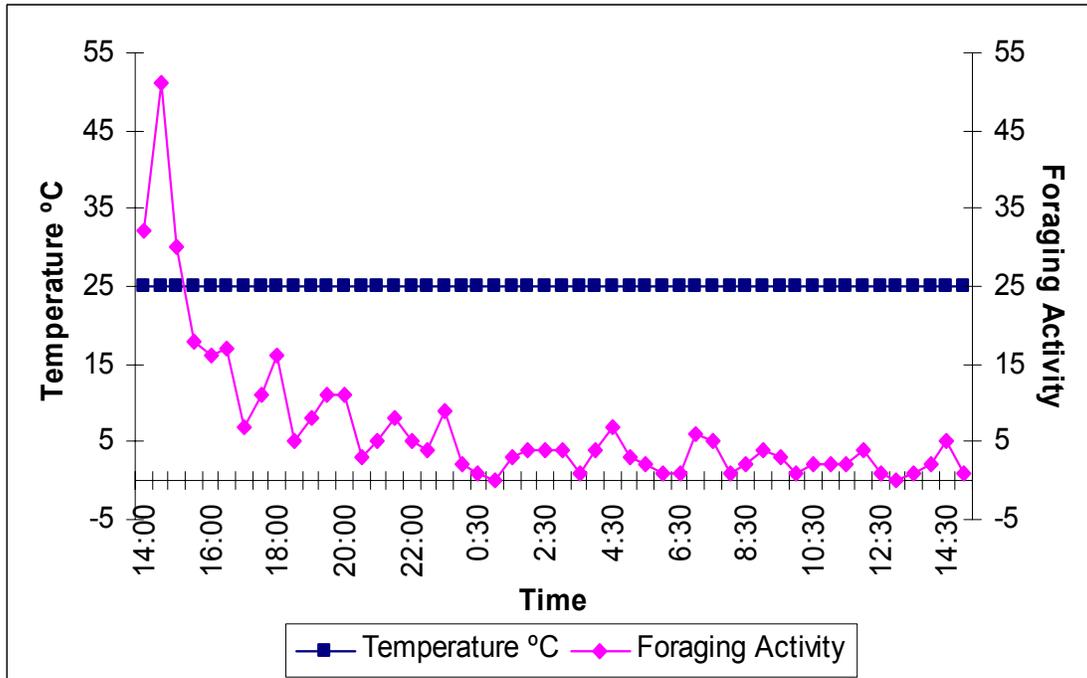


Figure 8: Twenty-four hour foraging activity of *Tapinoma sessile* in June 2001. A field colony was located and brought back to the laboratory. Ants were baited to a feeding station containing 20% sucrose solution and foraging activity was recorded using time-lapse photography. Temperature was maintained at approximately 25 °C.

When the laboratory foraging data for June were analyzed using trigonometric regression, the initial rush of foragers to the food source was not considered in the analysis. However, no strong correlation between temperature, time and foraging activity was observed (**Figure 9**).

In July, a new colony of odorous house ants (colony 7) was located and followed. This colony was a very large colony that numbered in the thousands of individuals. The colony showed relatively strong foraging activity throughout the day and night (**Figure 6c**). Temperatures never fell below 19 °C and average temperatures for the month of July were generally higher than in spring (April and May). There was a gradual increase in the number of foragers active at the feeding station for the first twelve hours of the study. After approximately 7:00, activity began to decrease even as temperatures started to rise. Activity finally declined at the end of the study with the onset of rain. The regression analysis of colony 7 (**Figure 7**) indicated that foraging was inversely affected by time and temperature when compared to what was observed in April and May. More foragers were active in the evening and early morning when temperatures were generally lower.

No data were collected in August due to inclement weather. However, a very strong and active colony (colony 8) was observed in September foraging during all times of the day and night. Activity patterns for this colony were similar to those recorded in July (**Figure 6d**). September foraging began with a peak of activity and then a general decline in foraging with decreasing temperatures for the first twelve hours of the study. However, unlike data collected in April and May, foraging activity increased sharply at approximately 2:00 when temperatures dropped to approximately 12 °C. This increase in foraging activity continued for approximately seven hours. At 13:00 when temperatures peaked at over 40 °C, foraging activity began to gradually decrease. After which, temperatures dropped at the end of the study and foraging activity began to slightly increase by 14:30. The regression analysis for colony 8 in September indicated a greater percentage of foragers more active during the time of the day when temperatures were relatively cool (**Figure 7**). The foraging activity in September was similar to what was observed in July.

Odorous House Ant Foraging Activity – June Laboratory Trial 2001

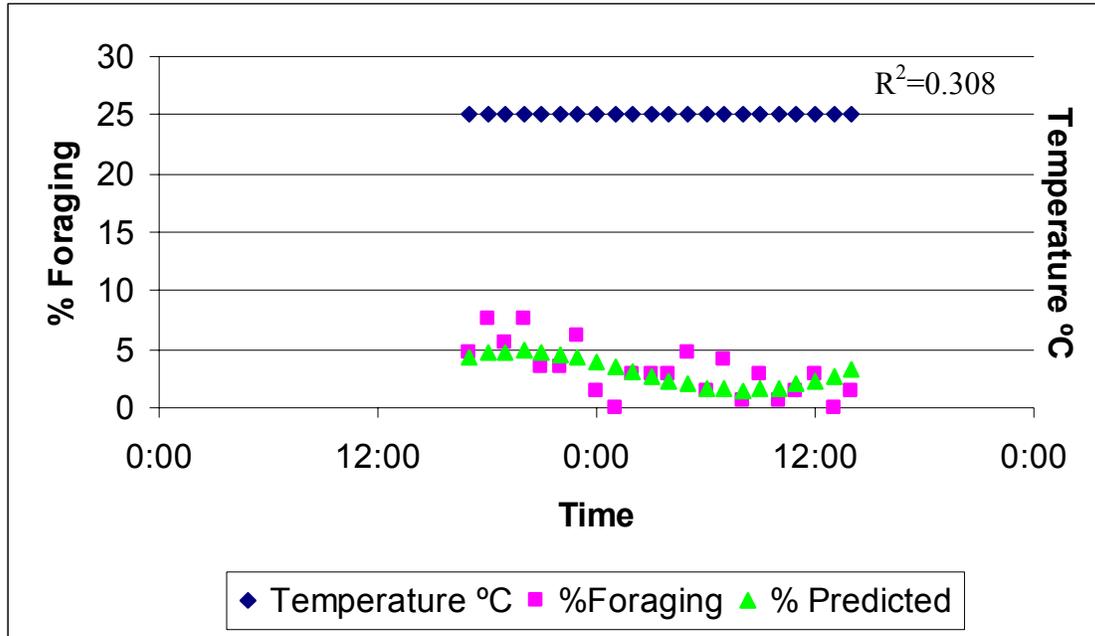


Figure 9: Twenty-four hour foraging activity of *Tapinoma sessile* in June 2001. A field colony was brought back to the laboratory and foraging activity was recorded using every hour for twenty-four hours was recorded. Ants were baited to a feeding station containing 20% sucrose solution. P-value = 0.030. No strong correlation was found between time, temperature °C and foraging activity when temperature was held constant at approximately 25 °C.

3.3.3 Initial Food Preference Tests

Initial food preference studies conducted in August of 2000, with carbohydrate, protein and lipid food samples indicated a significant preference for sugars over proteins and lipids (**Figure 10**). Casein hydrolysate and lipids were consumed less, but not completely ignored by the ants. Therefore, these macronutrient food items, plus non-hydrolyzed casein were chosen for the seasonal food preference tests. Non-hydrolyzed casein was also tested to record ant responses to a non-hydrolyzed protein.

3.3.4. Seasonal Food Preference Tests

In May, 2001 all test samples were consumed to some extent (**Figure 11**). Consumption of sucrose was significantly greater than that of casein hydrolysate, casein and lipids. In addition, no differences were found in the consumption between non-hydrolyzed casein and the lipids tested.

The consumption of sucrose in June 2001 was significantly greater than non-hydrolyzed casein and lipid samples. No significant differences in consumption were found between sucrose and casein hydrolysate (**Figure 12**). The lipid samples were consumed significantly less than sucrose. Total consumption of sucrose and casein hydrolysate decreased in June compared to May. However, non-hydrolyzed casein and lipid samples were consumed in slightly greater quantities than in May.

The results of the July 2001 tests were somewhat similar to the June results. A slight increase in the consumption of casein hydrolysate was observed when compared to June results. No significant differences were observed between sucrose, and casein hydrolysate. Sucrose and casein hydrolysate were consumed in significantly greater amounts than casein and the lipid sample. No differences in consumption were found in lipid and non-hydrolyzed casein samples (**Figure 13**).

In almost all trials, sucrose and casein hydrolysate samples were consumed in significantly greater amounts than any other sample tested. Lipid samples were consumed the least. However, in August 2001, colonies consumed carbohydrate, protein, and lipid samples in equivalent amounts. In these tests, no significant differences in consumption were detected among the test diets (**Figure 14**).

Odorous House Ant Food Preference Test – August 2000

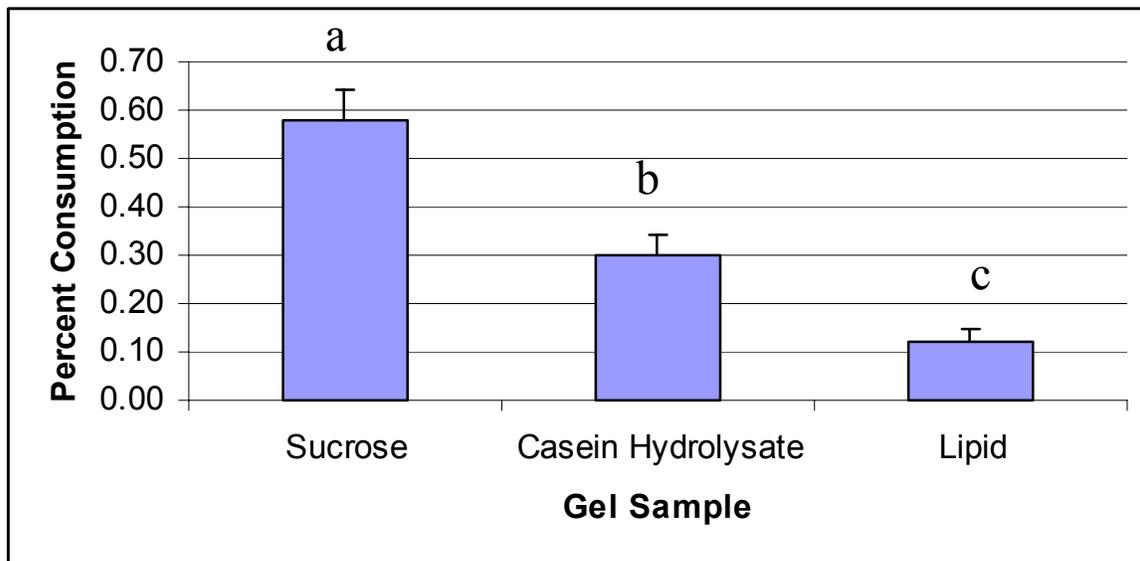


Figure 10: Mean consumption (as a percent of total colony consumption) in August 2000 of 20% sucrose, 5% casein hydrolysate and a lipid sample consisting of the combination of 2.5% palmitic acid and 2.5% extra virgin olive oil. Gel samples were presented to odorous house ants over a 24-hour period. Means with the same letter are not significantly different. $N=9$, $P = 0.0001$, vertical bars = \pm SEM

Odorous House Ant Food Preference Test – May 2001

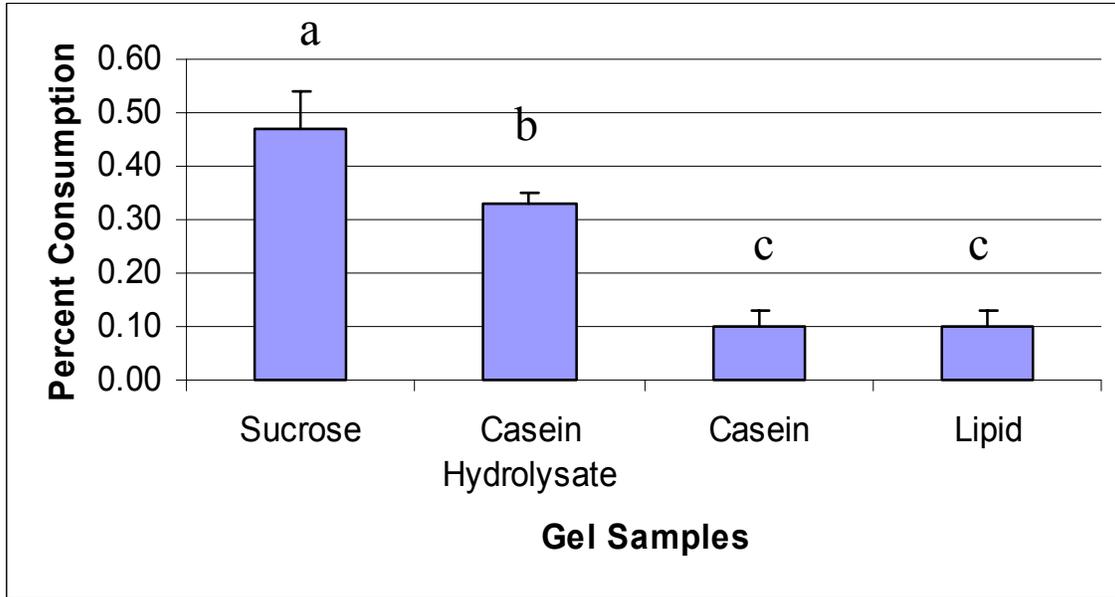


Figure 11: Mean consumption (as a percent of total colony consumption) in May 2001 of 20% sucrose, 5% casein hydrolysate, 5% casein and a lipid sample consisting of the combination of 2.5% palmitic acid and 2.5% extra virgin olive oil. Gel samples were presented to odorous house ants over a 24-hour period. Means with the same letter are not significantly different. $N=10$, $P=0.0001$, vertical bars = \pm SEM.

Odorous House Ant Food Preference Test – June 2001

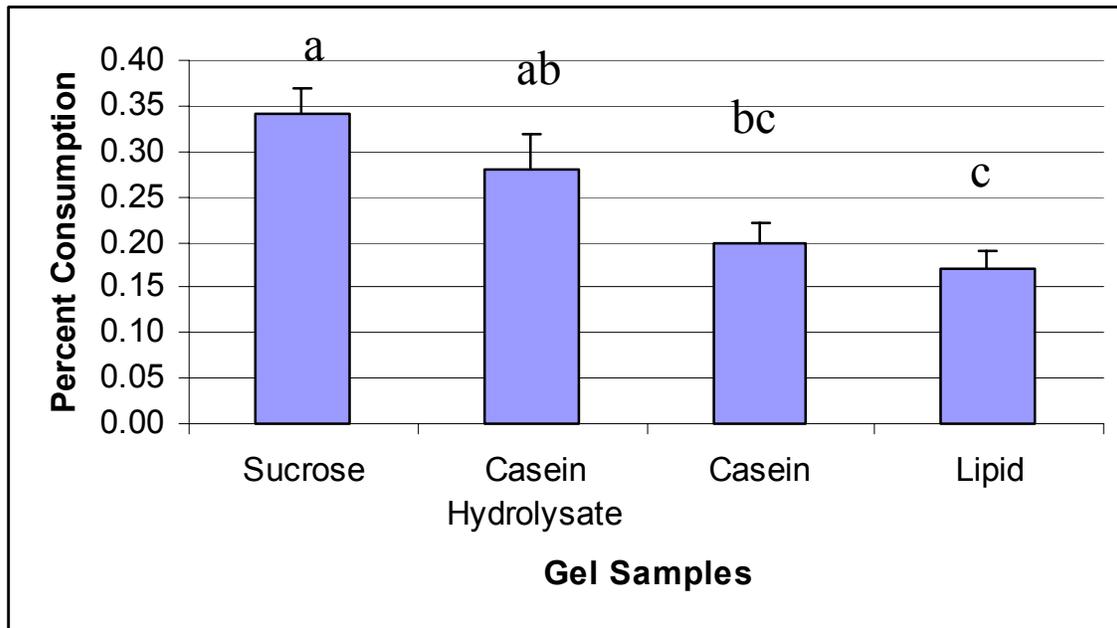


Figure 12: Mean consumption (as a percent of total colony consumption) in June 2001 of 20% sucrose, 5% casein hydrolysate, 5% casein and a lipid sample consisting of the combination of 2.5% palmitic acid and 2.5% extra virgin olive oil. Gel samples were presented to odorous house ants over a 24-hour period. Means with the same letter are not significantly different. $N=7$, $P=0.0015$, vertical bars = \pm SEM.

Odorous House Ant Food Preference Test – July 2001

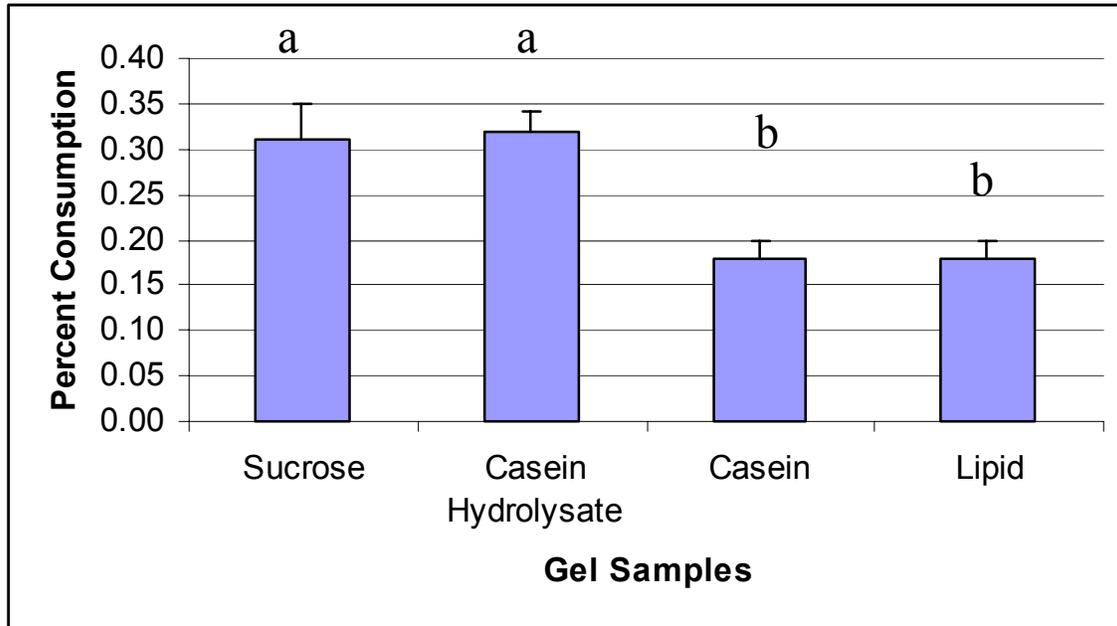


Figure 13: Mean consumption (as a percent of total colony consumption) in July 2001 of 20% sucrose, 5% casein hydrolysate, 5% casein and a lipid sample consisting of the combination of 2.5% palmitic acid and 2.5% extra virgin olive oil. Gel samples were presented to odorous house ants over a 24-hour period. Means with the same letter are not significantly different. $N=8$, $P=0.0002$, vertical bars = \pm SEM.

Odorous House Ant Food Preference Test - August 2001

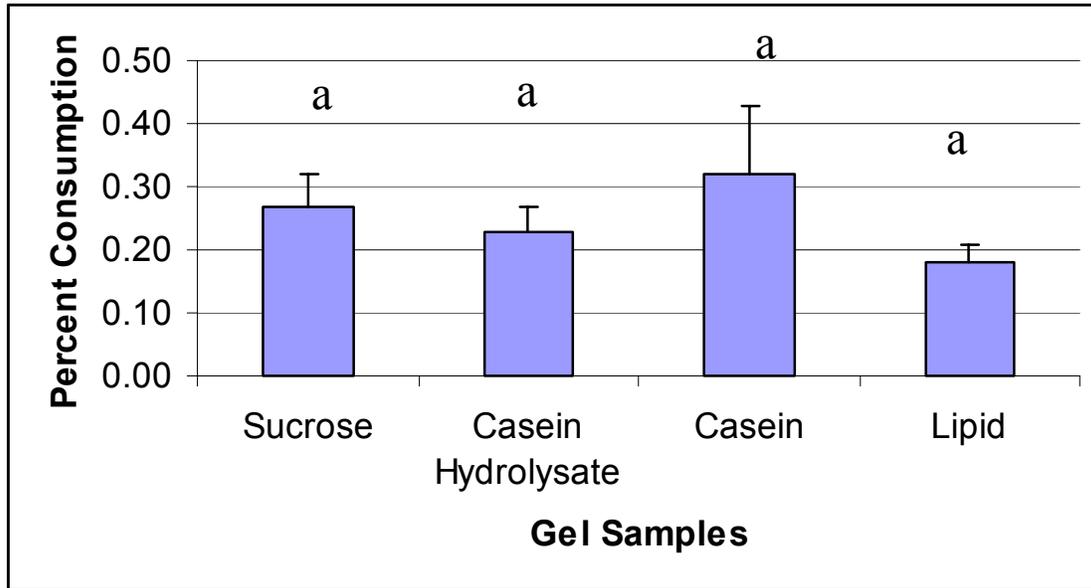


Figure 14: Mean consumption (as a percent of total colony consumption) in August 2001 of 20% sucrose, 5% casein hydrolysate, 5% casein and a lipid sample consisting of the combination of 2.5% palmitic acid and 2.5% extra virgin olive oil. Gel samples were presented to odorous house ants over a 24-hour period. Means with the same letter are not significantly different. $N=6$, $P = 0.5038$, vertical bars = \pm SEM.

3.4 Discussion

The purpose of this study was to gain a better understanding of odorous house ant seasonal foraging activity and seasonal food preferences. In general, odorous house ants do not discriminate between the time of day or night while foraging. When an acceptable food source is encountered, foraging trails are immediately established to the source and foraging continues throughout the day and night until the resource is depleted or is no longer desirable. However, it was observed that odorous house ants have a tendency to forage in greater numbers when temperatures are above 10 °C in the spring, and during the cooler diurnal temperatures in the summer months.

Colonies emerging from the overwintering stage in spring were relatively small. In April and May, foraging activity was generally restricted to temperatures that ranged between 15 and 30 °C. Odorous house ants did not forage when temperatures were below 10 °C. However, in July and September, most of the foraging activity was observed during the evening and early morning when temperatures ranged between 12-14 °C. At some of the test sites during July and September, temperatures reached above 40 °C. At these sites, odorous house ants showed only a slight decrease in foraging activity. When temperatures were maintained at approximately 25 °C in the laboratory, ant foraging activity was continuous and no distinct peaks or foraging patterns were observed.

Foraging activity during the summer months when the colony populations were greater in number did not seem to be as affected by relatively lower temperatures. During the summer months, foraging activity was observed at almost any time of day and night. However, odorous house ants did show a tendency to forage in greater numbers during times of the day and night when temperatures were cooler. The foraging activity during the cooler times of the day was the opposite of what was observed during the spring; ants foraging during the warmer times of the day. The odorous house ants also did not demonstrate distinct peaks of foraging activity like those of the black carpenter ant *C. pennsylvanicus*, whose peak foraging activity is nocturnal (Klotz, 1984; Cannon, 1998).

Trigonometric periodic regression analysis provides an alternative method of analyzing circadian data. This method of analysis can be used to predict the percentage of ants foraging given a specific month, at a particular time and temperature. P-values for all foraging activity tests were highly significant. The regression analyses indicated similar foraging activity patterns in April and May. The foraging patterns observed in spring were reversed during the summer months (July and September); more foraging activity during cooler temperatures.

Average temperatures for the Blacksburg area were recorded each month and compared to a thirty-year average. Temperatures during spring and summer months of 2001 did not produce unusual weather patterns and fell within one or two degrees of the thirty-year average.

The overall results from seasonal food preference tests indicate that when given a choice, odorous house ants preferred sucrose to the protein and lipid materials tested. In some trials the ants consumed the entire sample containing sucrose. The odorous house ants also consumed a substantial amount of the casein hydrolysate. Non-hydrolyzed casein, which contains approximately 90% protein, was consumed to some extent but was not as attractive to the ants as the sucrose and casein hydrolysate. It is likely that the odorous house ants responded to the free amino acids in the casein hydrolysate as opposed to proteins found in non-hydrolyzed casein.

In June, July and August 2001, the combined consumption of protein materials (casein plus casein hydrolysate) was higher than total sucrose consumption (**Figure 15**). The increased need for protein may be due to increased brood production during the summer, and to the production of reproductives. Colonies were also larger during the summer and the ants foraged throughout the day and night.

Seasonal food preference tests showed slight differences between the consumption of lipids and casein hydrolysate. Lipids were never consumed in greater

amounts than casein hydrolysate and in no case were individual lipid, casein and protein samples ever consumed in greater amounts than sucrose.

In addition, seasonal food preference tests indicate that odorous house ants would feed continuously on sugary foods throughout the spring and summer. From April to August 2001, no significant seasonal shifts in food preference were observed among the four macronutrient food samples tested. When given a choice, sucrose was always the preferred food item, even though *total* protein consumption did increase during late summer.

During the course of our investigations into the feeding preferences of *T. sessile*, several unexpected observations were recorded with respect to ant behavior. When odorous house ants discover a preferred food source, they covered the food item with debris collected from the surrounding environment. This behavior was also observed in the laboratory where odorous house ants were observed to fill the entrance of the sample tube with bits of debris, barricading foraging workers inside (**Figure 16**). The workers inside the tube continued to forage and collect food. This behavior may represent an attempt to protect, or camouflage a food source from potential competitors. However, despite their efforts to protect a food source, most field colonies we observed were easily displaced by other competing ant species such as honey ants or acrobat ants.

Percent Consumption of Nutrients by Odorous House Ants

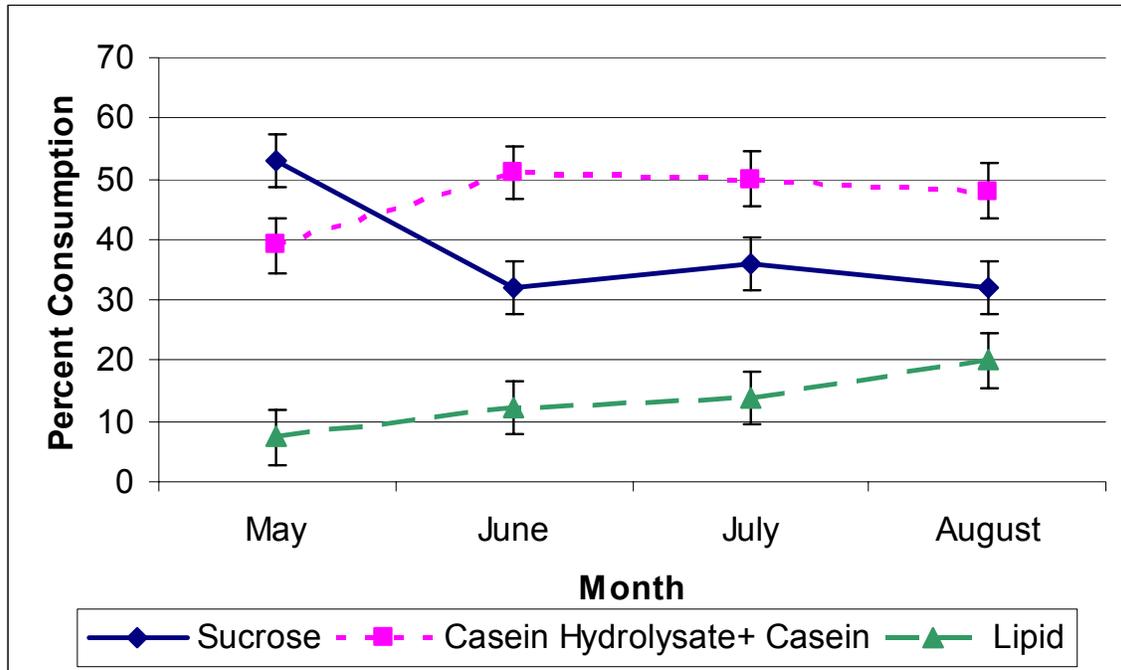


Figure 15: Total percent consumption of nutrient samples. Samples consisted of: 20% sucrose, 5% casein hydrolysate, 5% casein and a lipid sample consisting of the combination of 2.5% palmitic acid and 2.5% extra virgin olive oil. Gel samples were presented to colonies of *Tapinoma sessile* in May, June, July and August 2001 over a 24-hour period.



Figure 16: Odorous house ants were offered a variety of food samples to determine food preferences. Food samples were formulated into a gel matrix and placed into plastic microcentrifuge tubes. The ants were allowed to feed on the sample for up to twenty-four hours. After testing preferences were determined by measuring consumption. Odorous house ants were observed filling the entrance of the plastic microcentrifuge tubes containing preferred food items. The ants blocked the entrance to the tube with bits of debris, barricading foraging workers inside.

Although other ant species may indeed demonstrate seasonal feeding preferences for certain types of foods (Cannon, 1998), odorous house ants did not. These findings are consistent with Bistows' (1984) research on the benefits of ant attendance to honeydew producing homopteran insects. Bristow concluded no clear pattern of seasonal feeding preference was found for protein or carbohydrate in *T. sessile* or the myrmica species investigated.

Wang and Brook (1972) and Smith (1928), also conducted biological studies on the odorous house ant. Smith found that under normal conditions odorous house ants are largely honeydew loving insects. Smith also reported accounts of odorous house ants entering homes and feeding on sweet foods, but noted that they would feed on protein sources such as: uncooked beef, fish, cheese, and milk. These reports support our findings that show a strong feeding preference for sweet foods with the additional

consumption of proteinaceous foods during the spring and summer months. Wang and Brook (1972) concluded that odorous house ants were mostly attracted to sugary rewards such as syrup and apricot and blackberry jam. Wang and Brook's results do not indicate specific nutrients preferences such as sugar or protein types, nor do they indicate preferences among varying food concentrations.

Argentine ants (*Linepithema humile* (Mayr)) are similar to odorous house ants and are classified in the same subfamily, Dolichoderinae. Like the odorous house ant, the Argentine ant is considered to be a house-infesting pest. Baker et al. (1985) showed that Argentine ants preferred liquid, sugary foods to all other types of food; in particular 25% sucrose solution and 25% honey water. The preference for sugar rewards in Argentine ants is similar to what was observed in this study. However, Baker et al. (1985) found that the addition of casein hydrolysate to sucrose water, reduced the feeding response of Argentine ants. This response to casein hydrolysate is contrary to what was observed in this study with odorous house ants.

Though more tests need be conducted to better define seasonal food preferences of *T. sessile*, it is clear that they are primarily attracted to "sweet" liquid foods such as sucrose in a manner similar to what has been described for the Argentine ant (Hedges, 1998 and Baker, et al., 1985). *T. sessile* is considered to be a major pest species in many parts of the United States and can be difficult to control once established in an urban setting. Understanding its foraging and feeding behaviors may prove useful for future development of more effective control methods and ant management programs.

CHAPTER 4: SPECIFIC FOOD PREFERENCES OF THE ODOROUS HOUSE ANT (*Tapinoma sessile* (SAY))

4.1 Introduction

Since the mid 1920's, the odorous house ant (*Tapinoma sessile*) has been an important urban pest in many regions of the United States. In recent years, this house-infesting species has become an even greater problem and is now considered to be the number one call-back pest in many parts of Virginia. Colonies of odorous house ants can number in the hundreds of thousands of individuals and once established in an urban setting are difficult to control.

The traditional approach to control is to use an insecticidal spray formulation. This control method is not very effective and can often exacerbate problems by causing colonies to split into several satellite colonies. However, before a more effective method of control can be developed, a better understanding of the basic biology of the odorous house ant is needed.

Currently, little is known about specific food preferences of *T. sessile*, except that they consist primarily of sweet foods such as honeydew, and dead insects (Smith, 1965). Past reports of food preferences are composed mainly of historical anecdotes and unsystematic observations of feeding on complex food items and processed foods (jams, jellies and meat grease). For example, Wang and Brook (1972) tested feeding preferences of the odorous house ant by offering such foods as: apricot jam, blackberry jam, apple butter, meat grease, cottonseed oil, orange juice and syrup. Their results suggested that odorous house ants were mostly attracted to syrup, apricot jam and blackberry jam. Unfortunately, their results do not indicate specific macronutrient preferences, such as sugar or protein types that attract foraging ants, nor do they reveal preferences among varying concentrations of food types.

The purpose of this study was to investigate specific macronutrient food preferences of the odorous house ant (*Tapinoma sessile*) with the idea that this knowledge may prove useful in the future development of more effective control methods.

4.2 Materials and Methods

4.2.1 Collecting and Maintaining *Tapinoma sessile* Colonies

Colonies of *T. sessile* were located around the Blacksburg area in Montgomery County, Virginia. Once the colonies were located, they were transferred into 18-quart plastic containers and brought back to the Dodson Urban Pest Management Research Laboratory at Virginia Polytechnic Institute and State University for study under more controlled conditions. Temperature inside the insect rearing room where colonies were kept, was maintained at $25^{\circ}\text{C} \pm 3^{\circ}\text{C}$. Colonies were provided with hard wood mulch as a substrate and nest material, and with water filled vials fitted with a cotton wick for continuous access to water. A spray bottle was used daily to mist water into the containers to keep the environment moist. Mineral oil was used to coat the inner surface of the containers to prevent escape and all colonies were maintained on a diet that consisted of a 20% sugar solution and a supply of dead insects.

4.2.2 Food preference assays

Specific food preference tests were conducted using laboratory colonies as described in **Section 4.2.1**. The colonies were allowed to acclimate for approximately 3 days after which testing was initiated. The colonies were also starved for at least 2 days prior to each test; food was withheld and only water was provided. The food preference tests consisted of offering the ants a choice among gel or liquid food samples. The food samples consisted of a variety of carbohydrates (simple sugars), lipids, salts and/or protein containing materials at varying concentrations.

Carbohydrates. Preliminary tests were conducted to identify specific sugar preferences. Sugars commonly found in the ant's environment were tested and included: sucrose, glucose, fructose, maltose and trehalose. The individual sugars were prepared in distilled water at concentrations ranging from 3% to 30%. Aliquots of the sugar solution were transferred into 0.5ml plastic centrifuge tubes (Fisher Scientific) for testing. Tubes were labeled, sealed and stored in the refrigerator at approximately 3.8° C. In these tests, control tubes consisted of distilled water.

Sucrose samples were also tested in a gel and used as a base matrix for testing other food items. Sugar solutions were mixed with agar to yield a gel matrix with a final concentration of 15% sucrose. The agar gel was prepared by boiling 10ml of distilled water and slowly adding 0.24g of agar (Sigma Chemical Company) to the boiling water while stirring. In a separate beaker, 3.6g of sucrose (Sigma Chemical Company) were dissolved in 10ml of distilled water. When the agar was completely dissolved, the sucrose solution was slowly added to the agar while stirring. Aliquots of this mixture were transferred into 0.5ml plastic centrifuge tubes (Fisher Scientific) and placed directly into an ice bath for rapid cooling. When the samples gelled, the tubes were labeled, sealed and stored in the refrigerator at approximately 3.8° C. Control tubes consisted of only agar gel.

Lipids. Lipid samples were formulated with 15% sucrose in an agar gel matrix to examine feeding responses of the ants to different lipid materials. The lipids tested were extra virgin olive oil, cod liver oil (Marquee Brand) and menhaden oil (Omega Protein™) at concentrations ranging from 1% to 5%. Previous studies evaluating lipid consumption in different ant species have shown extra virgin olive oil to be either non-repellent or slightly attractive due to relatively low concentrations of oleic acid (Fell, unpublished). Cod liver oil and menhaden oil are malodorous fish oils that consist of a blend of different fatty acids. Cod liver oil is mainly composed of approximately 6% miristic, 10-20% palmitoleic, 25% oleic and 45% araquidonic and clupanodonic acids (Bionatus website, 2002). Major acids commonly found in menhaden oil include: 15% palmitic acid, 11% oleic acid, 10% palmitoleic acid (Omega website, 2001).

The lipid samples were prepared in a similar manner to the carbohydrate gel samples except that they were made up as emulsions. The lipid sugar gels were prepared by dissolving 15.2g of sucrose in 100ml of distilled boiling water and then slowly adding 1.2g of agar while stirring. Aliquots of 1.35g, 0.657g or 0.27g of olive oil were then combined with 25ml of the sucrose agar solution to yield final olive oil concentrations of 5%, 2.5% and 1% respectively. The emulsions were transferred into 0.5ml plastic centrifuge tubes sitting in an ice bath to rapidly cool the mixture and maintain the lipid suspension. When the samples gelled, the tubes were labeled, sealed and stored as previously described. This procedure was repeated using cod liver oil and menhaden oil. Control tubes consisted of agar gels containing 15% sucrose.

Proteins. Lactalbumin hydrolysate (Bio-Serv), brewer's yeast hydrolysate (Bio-Serv) and casein acid hydrolysate from bovine milk (Sigma Chemical Company) were used in the protein preference tests. In previous studies, both casein and casein hydrolysate have produced good feeding responses with different species of ants (Cannon, 1998).

Casein hydrolysate samples were prepared as previously described using 0.24g of agar in a total of 20ml of distilled water and 1.07g of casein hydrolysate to obtain a concentration of approximately 5% protein material (by weight). These samples were formulated in 15% sucrose gels to obtain maximum feeding responses and were prepared as previously described. Brewer's yeast and lactalbumin hydrolysate were prepared at 5% concentrations in a manner similar to the casein hydrolysate. However, lactalbumin was also formulated at varying concentrations ranging from 1-5%. Control tubes consisted of 15% sucrose in agar and/or plain agar gel.

Amino Acids. In addition to protein containing materials, 20 L-amino acids (**Table 1**) were also used in food preference tests. It has been shown that gustatory chemoreceptors of many insects will respond to amino acids (Chapman, 1995). Therefore, each amino acid tested was prepared as a 100mM solution in distilled water

and control tubes consisted of distilled water. Insects may have different amino acid requirements in their diet so it was important to test a variety of amino acids (**Table 1**).

Salts. Sodium chloride (NaCl) (Sigma Chemical Company) test samples were made by first preparing a 15% sucrose solution with 1% NaCl. Additional solutions of 0.75%, 0.5% and 0.25% NaCl were prepared by serial dilution. In tests performed by Fell (unpublished) on *Camponotus pennsylvanicus*, NaCl was shown to increase consumption rates in test samples. All samples were labeled, sealed and stored as previously described. Control tubes consisted of 15% sucrose solution.

Test Procedures. Gel sample tubes were weighed gravimetrically immediately before testing using an analytical balance XA-200DS (Fisher Scientific). The analytical balance was calibrated using standard weights; the error was less than 1%. The tubes were placed in a random array inside containers housing odorous house ants (**Chapter 3**). Ants were allowed to feed at the sample tubes for up to 24 hours after which the tubes were removed and the final weights (mg) recorded. Preferences were determined by measuring consumption. Because odorous house ants are mainly liquid feeders, liquid samples were tested for either 20 minutes or 12 hours depending on the samples and the rate of consumption. Consumption was converted to percent consumption to account for differences in colony size.

Table 1: List of twenty amino acids used to test feeding responses in 3 laboratory colonies of *Tapinoma sessile* in August 2001. Each amino acid was prepared at a concentration of 100mM solution in distilled water. Control tubes consisted of distilled water. Amino acids followed by an “E” are considered essential (Chapman, 1998).

Amino Acids Tested	
Alanine	Leucine (E)
Arginine (E)	Lysine (E)
Asparagine	Methoionine (E)
Aspartic Acid	Phenylalanine (E)
Cysteine	Proline
Glutamic Acid	Serine
Glutmine	Threonine (E)
Glycine	Tryptophan (E)
Histidine (E)	Tyrosine
Isoleucine (E)	Valine (E)

Amino Acid Test Procedures. The twenty amino acid samples were randomly divided into 3 groups of 7 plus distilled water as a control. The amino acid samples were transferred into 200ul capillary tubes (Fisher Scientific) and the initial weight (g) of each sample tube was recorded. The capillary tubes with the different amino acid solutions were arranged in a random array on a petri dish inside a foraging arena. The capillary tubes were secured to the rim of the petri dish with modeling clay (**Figure 17**). Three odorous house ant colonies were then connected to one of three foraging arenas using PVC piping (**Figure 18**). Colonies were given 24 hours to feed on one group of amino acids. After the 24 hour time period, samples were collected and final weights were recorded. The groups of 7 amino acids were then randomly rotated among the 3 colonies for 3 consecutive days allowing each colony equal exposure to all amino acid samples.

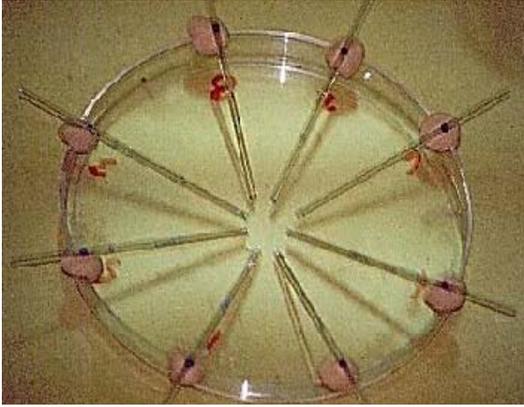


Figure 17: Random array of amino acids samples in capillary tubes fastened to the rim of dish using modeling clay.

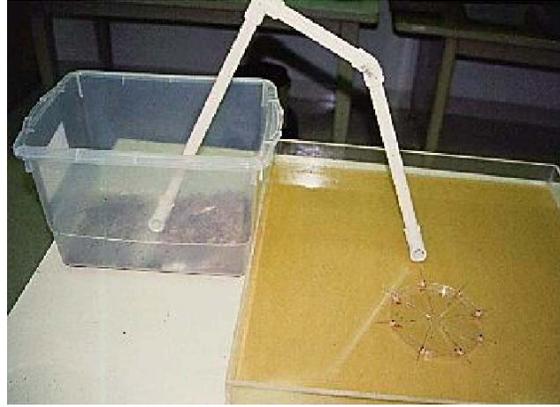


Figure 18: Experimental set up of a colony connected to a foraging arena.

4.2.3 Statistical Analysis

A modified randomized complete block design (I,IRRCBD) (Hinkelmann and Alcorn, 1998) was chosen as the model for data analysis using days as blocks. Data tested in one day were analyzed as a randomized complete block design using colonies as blocks. Seasonal food preference tests were analyzed using Analysis of Variance (SAS Institute, 1990). Values of $P \leq 0.05$ were used to indicate significance. Means were separated using Fisher's Protected Least Significant Difference. LS Means may have different variances; however in most of the analyses the variances were equal, hence the equal standard error for all treatments (Hinkelmann and Kempthorne, 1994).

4.3 Results

4.3.1 Macronutrient preferences

Macronutrient food preference tests showed that carbohydrates, proteins and lipids were all consumed to some degree by foraging odorous house ants. However, carbohydrates were generally preferred over proteins and lipids (**Chapter 3**). Moreover, the greatest consumption of sucrose solution occurred above 10% (**Figure 19**). In addition, 20% sucrose solution was preferred over the other sugars tested (**Figure 20**).

The addition of extra virgin olive oil to sample gels reduced consumption significantly in samples containing as little as 1% olive oil (**Figure 21**). The addition of cod liver oil produced similar results (**Figure 22**). Menhaden oil did not have a negative effect on consumption. Results indicate that odorous house ants did not strongly distinguish between control samples and samples containing the menhaden oil (**Figure 23**).

Sodium chloride, however, was found to have a positive effect on feeding responses in *T. sessile*. The addition of salt caused a significant increase in the consumption of sucrose samples that contained 0.5-1% sodium chloride (**Figure 24**).

Lactalbumin hydrolysate was also tested at varying concentrations. Odorous house ants did not distinguish between control samples and samples containing lactalbumin hydrolysate (**Figure 25**). Hydrolyzed brewer's yeast, another protein containing material, was tested against lactalbumin hydrolysate, casein hydrolysate and plain agar (**Figure 26**). These tests were conducted at the end of the season in late September and the data analysis showed no significant difference among the three protein containing materials, despite the fact that casein hydrolysate has shown positive feeding responses in *T. sessile* in earlier studies. More tests must be conducted using a larger sample size to better understand possible seasonal shifts and food preferences among the protein containing materials.

The initial amino acid preferences tests indicate that *T. sessile* may be able to discriminate among different amino acids and will feed on them to some extent. Phenylalanine, proline, alanine, threonine and glutamine was consumed in greater amounts than water (**Figure 27**). Further tests using a larger number of samples are needed to determine ant feeding responses to specific amino acids.

Odorous House Ant Food Preference Tests

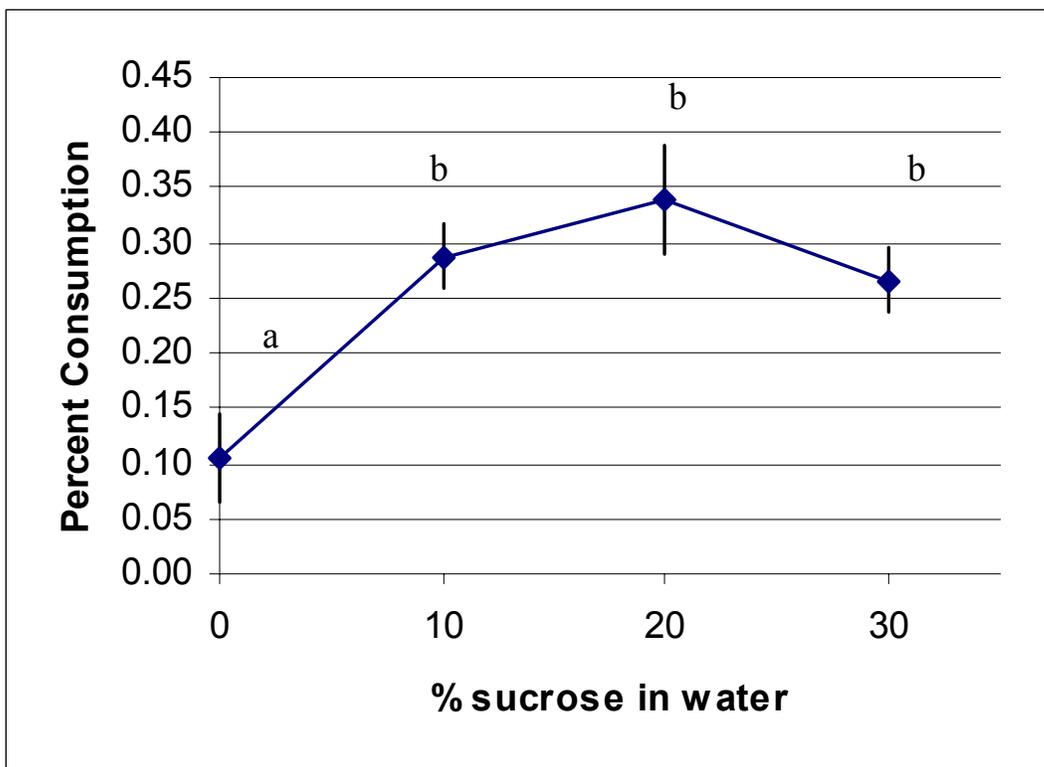


Figure 19: Mean consumption of varying concentrations of sucrose solution in August 2000 using laboratory colonies of *Tapinoma sessile*. Control = distilled water. ($P=0.004$) ($\alpha=0.05$) ($N=12$).

Odorous House Ant Food Preference Tests

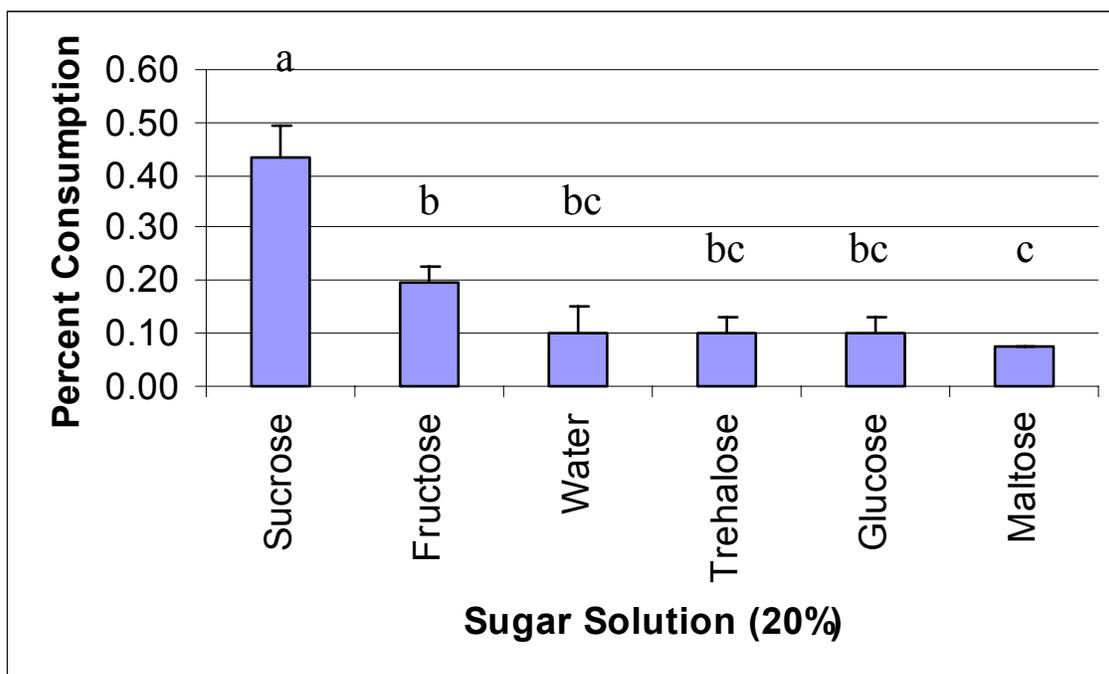


Figure 20: Mean consumption of 20% sugar solutions conducted in August 2000 using laboratory colonies of *Tapinoma sessile*. Control = distilled water. Means (\pm SEM) with the same letter are not significantly different. ($P=0.0001$) ($\alpha=0.05$) ($N=5$).

Odorous House Ant Food Preference Test

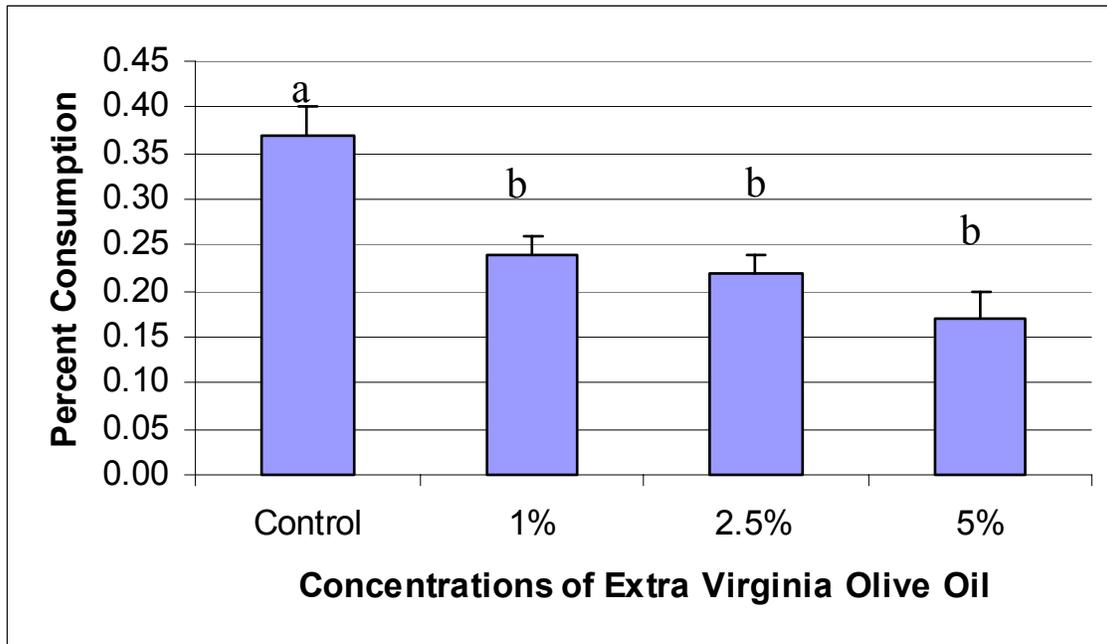


Figure 21: Mean consumption of gels containing varying concentrations of extra virgin olive oil in a gel matrix containing 15% sucrose. Choice test conducted in June 2001, using laboratory colonies of *Tapinoma sessile*. Control = 15% sucrose in gel matrix only. Means (\pm SEM) with the same letter are not significantly different. ($P= 0.0001$) ($\alpha= 0.05$) (LSD = 0.058) (N=6).

Odorous House Ants Food Preference Test

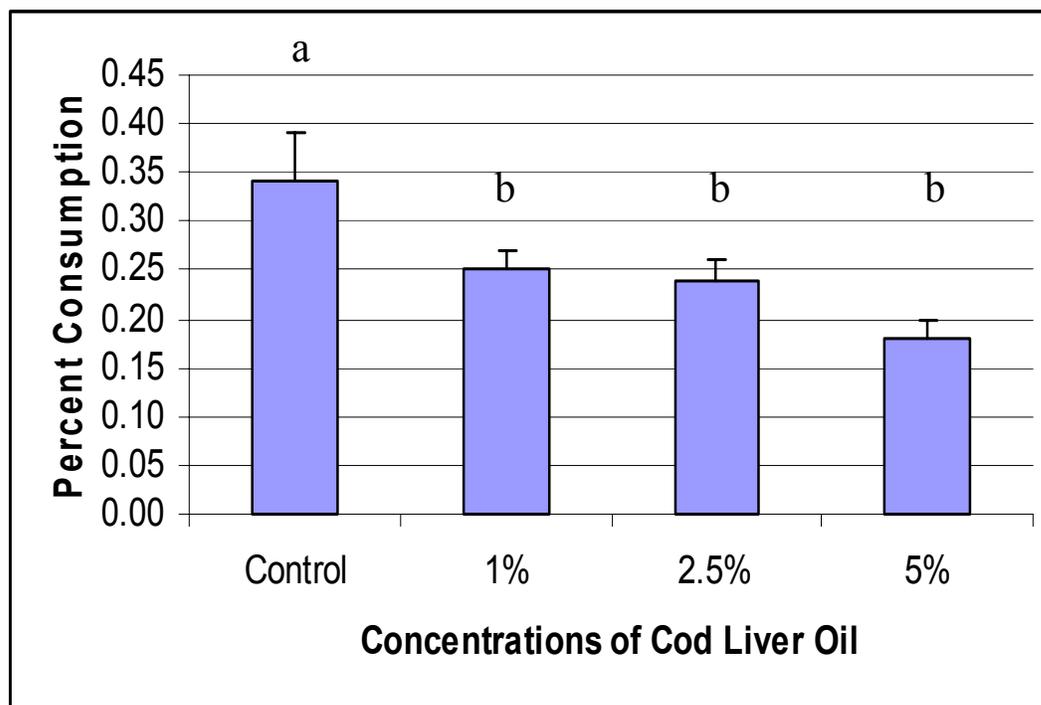


Figure 22: Mean consumption of varying concentrations of cod liver oil in a gel matrix containing 15% sucrose. Choice test conducted in June 2001 using laboratory colonies of *Tapinoma sessile*. Control = 15% sucrose in gel matrix only. Means (\pm SEM) with the same letter are not significantly different. ($P= 0.0089$) ($\alpha= 0.05$) (N=6).

Odorous House Ant Food Preference Tests

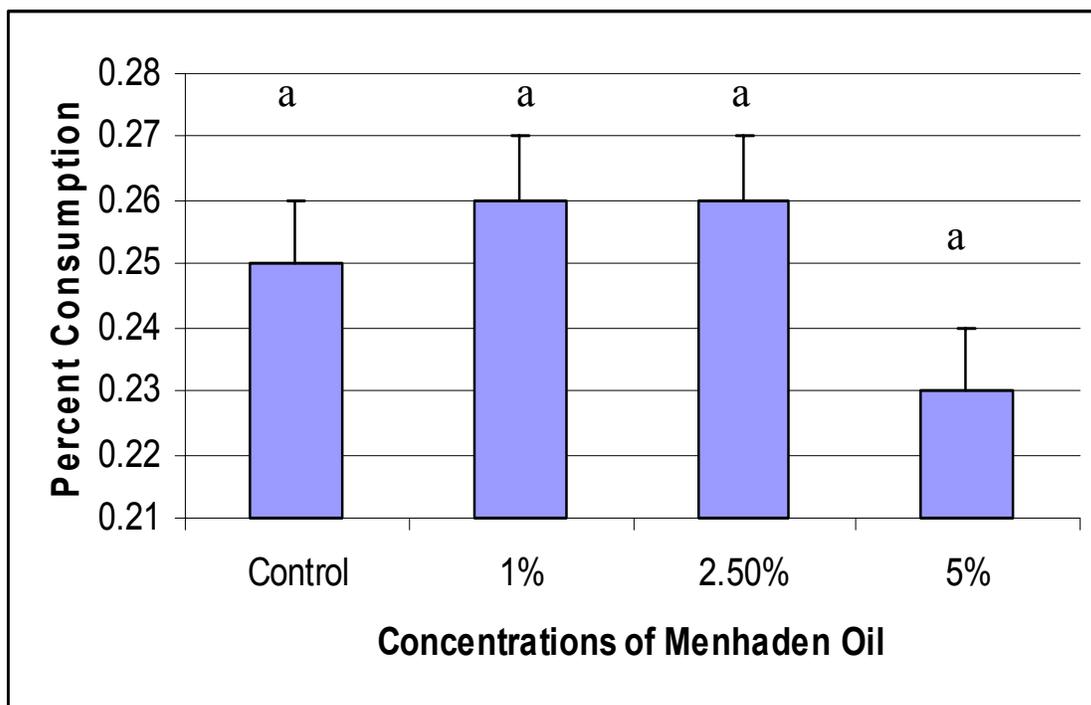


Figure 23: Total mean consumption of varying concentrations of menhaden oil in a gel matrix containing 15% sucrose. Choice test conducted in July 2001 using laboratory colonies of *Tapinoma sessile*. Control = 15% sucrose in gel matrix only. Means (\pm SEM) with the same letter are not significantly different. ($P= 0.2710$) ($\alpha= 0.05$) (N=14).

Odorous House Ant Food Preference Test

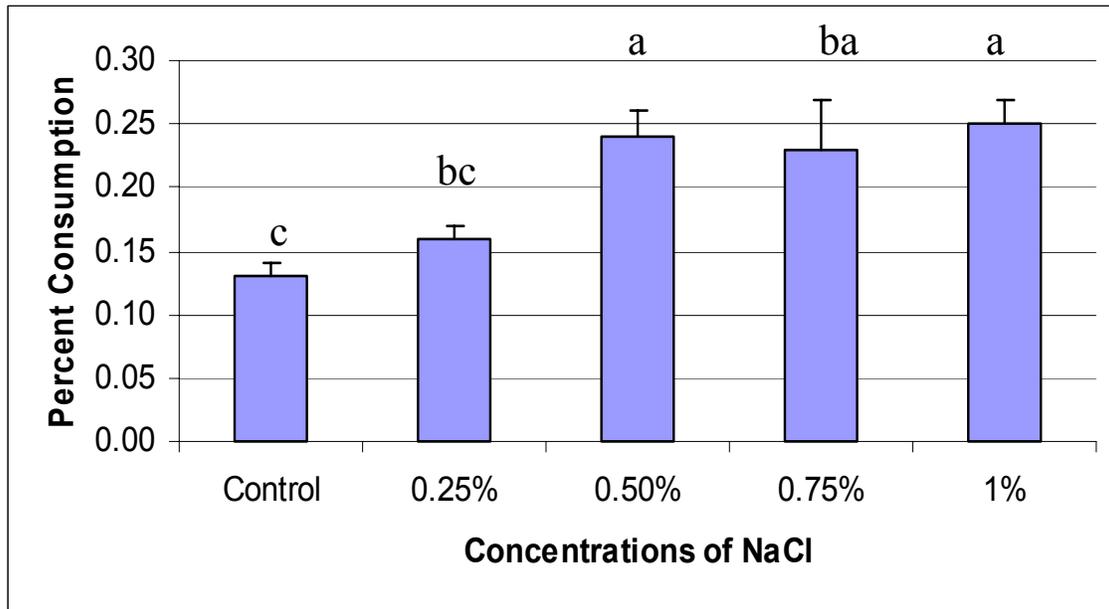


Figure 24: Mean consumption of varying concentrations of NaCl in a gel matrix containing 15% sucrose. Choice test conducted in July 2001 using laboratory colonies of *Tapinoma sessile*. Control = 15% sucrose in gel matrix only. Means (\pm SEM) with the same letter are not significantly different. ($P=0.007$) ($\alpha=0.05$) (N=6).

Odorous House Ant Food Preference Test

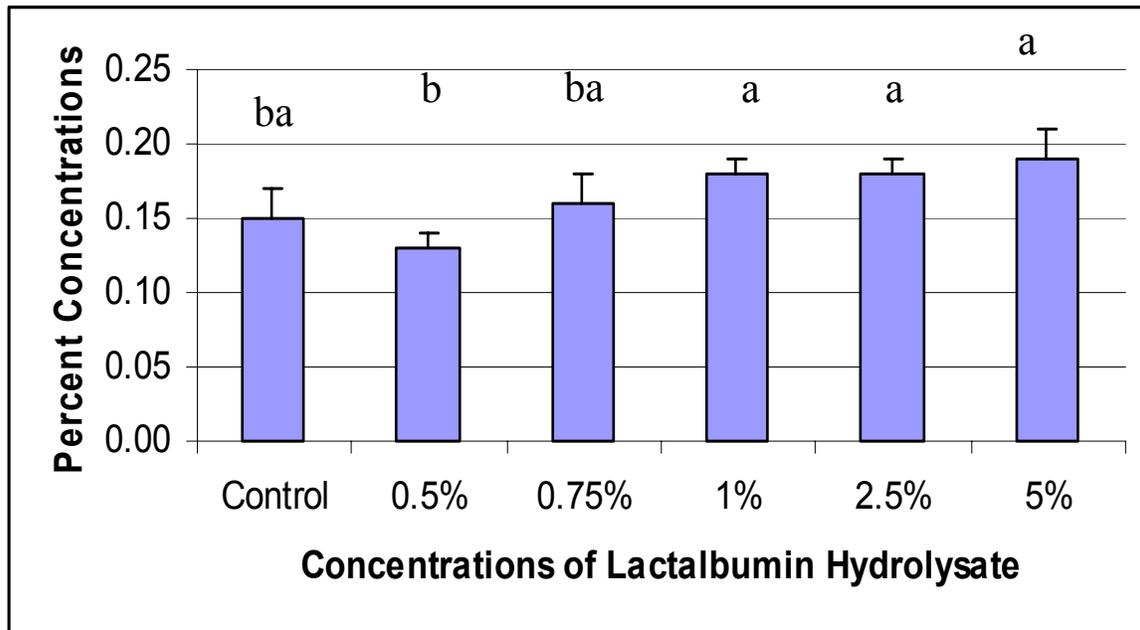


Figure 25: Mean consumption of varying concentrations of Lactalbumin Hydrolysate in a gel matrix containing 15% sucrose. Choice tests were conducted in July 2001 using laboratory colonies of *Tapinoma sessile*. Control = 15% sucrose in gel matrix only. Means (\pm SEM) with the same letter are not significantly different. ($P=0.08$) ($\alpha=0.05$) ($N=10$).

Odorous House Ant Food Preference Test

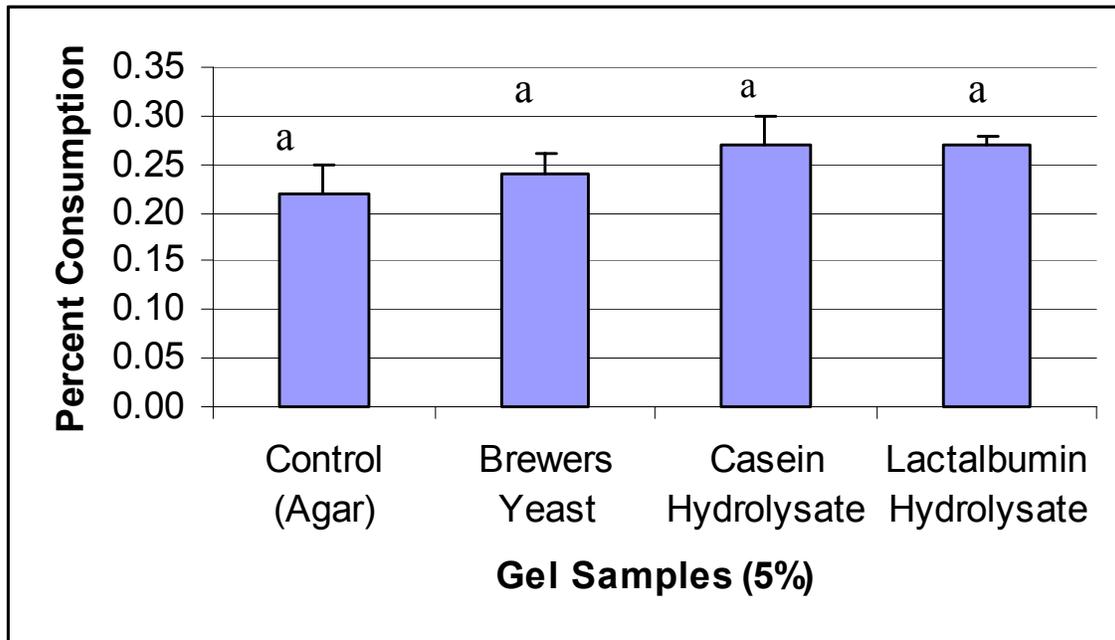


Figure 26: Mean consumption of various protein containing materials at 5% concentration in a gel matrix containing 15% sucrose. Choice tests were conducted in September 2001 using laboratory colonies of *Tapinoma sessile*. Control = Agar gel matrix only. Means (\pm SEM) with the same letter are not significantly different. ($P= 0.358$) ($\alpha= 0.05$) (N=6).

Odorous House Ant Food Preference Test

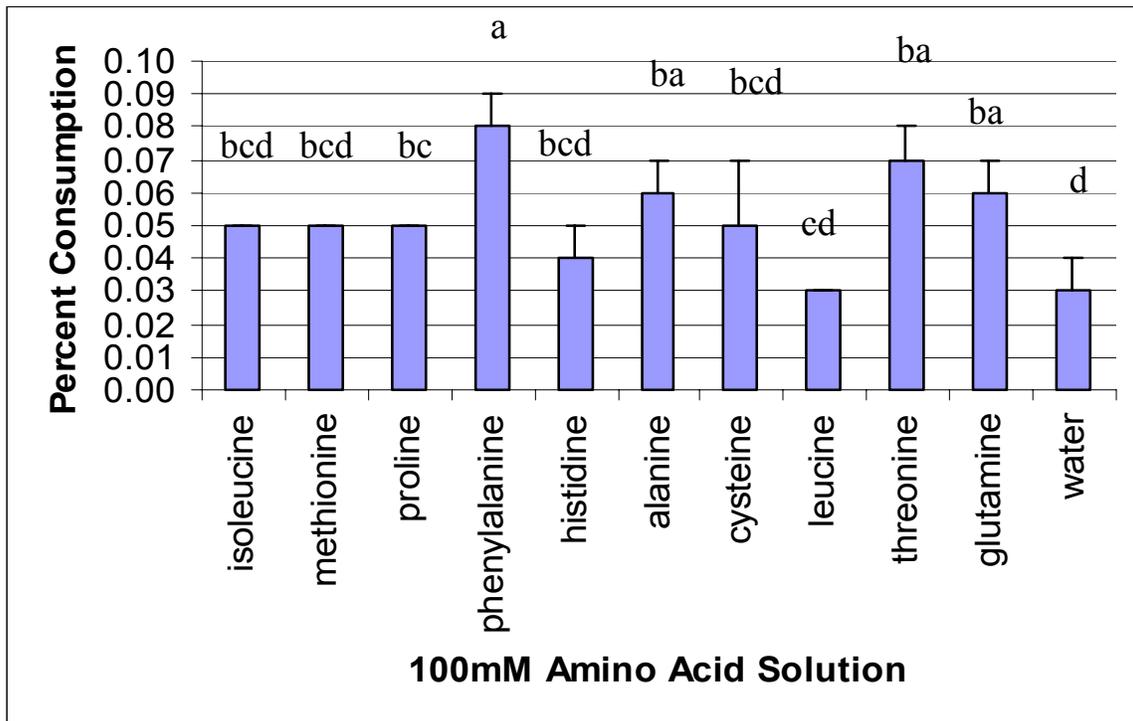


Figure 27: Mean consumption of 100mM amino acid samples conducted in August 2001 using 3 laboratory colonies of *Tapinoma sessile*. Control = d.H₂O. Means (\pm SEM) with the same letter are not significantly different. ($P=0.013$) ($\alpha=0.05$). Mean evaporation = 0.0127mg (N=3).

4.4 Discussion

In this study, the preference for sucrose by *T. sessile* was similar to that observed with carpenter ants (Cannon, 1998). Sucrose was preferred by *T. sessile* over the other sugars tested. Sucrose, fructose, plus glucose, are the three sugars most commonly found in honeydew (Fell and Morse, 1977). In addition, sucrose was generally favored at solution concentrations above 10%. Smith (1928) found *T. sessile* infesting many sweet foods that have high sucrose and fructose concentration such as: jellies, preserves, honey, sugar, pies, custards, ripe fruit and marmalades. Wang and Brook (1972) offered odorous house ants similar type foods and concluded that they were mostly attracted to syrup, apricot jam, and blackberry jam which also contain a high concentration of sucrose and fructose, but that they were not attracted to protein or lipid containing foods.

In addition to sugars, some ants will readily collect protein and lipid foods. Hooper and Rust (1997) investigated food preferences of the southern fire ant (*Solenopsis xyloni*) by incorporating a combination of readily available food products into a bait mixture. The original diet (described by Keller et al., 1989) contained beef hash as the primary source of protein. Hooper and Rust replaced this protein source with tuna, anchovy, sardine or mealworms. The diet was then baked or freeze-dried to formulate granules of varying particle size. The results indicated that diets containing anchovy were preferred over the other diet combinations. Although dried, powdered eggs were also found to be attractive.

Casein hydrolysate, a common milk protein, is known to be attractive to different species of ants (Cannon, 1998) and was also found to be highly acceptable to *T. sessile* at a 5% concentration. The ants were seen to respond better to hydrolyzed casein than the non-hydrolyzed form. In contrast, Becker, et al., (1985) found that the addition of casein hydrolysate to 25% sucrose caused a reduction to feeding in Argentine ants. Hydrolyzed casein is broken down into its constituent amino acids and some species of ants seem to react better where specific amino acids are available. This finding prompted an investigation into the feeding responses of *T. sessile* to different amino acids.

The contact chemoreceptors of many insects will respond to different amino acids. For example, in *Pieris barassicae*, fourteen amino acids were shown to stimulate cells in some sensilla of this lepidopteran larva, the most effective being histidine, phenylalanine and 4-hydroxyproline (Chapman, 1995). In contrast, histidine and phenylalanine were among the least stimulating for *Pieris rapae*, the cabbage butterfly (Chapman, 1995).

Amino acid preference tests have also been conducted by Ricks and Bradleigh (1970). Their results indicated that in general, the acceptance of amino acids by two species of *Solenopsis* was equal to or less than water. However, leucine was significantly more attractive than water. Leucine was also as acceptable as several test sugars. In addition, both species showed similar preferences for melezitose, sucrose and glucose, and rejected xylose, ribose, mannose arabinose and galactose. Vitamins were also tested and it was found that folic acid, B₁₂, and inositol were preferred over water. None of the B vitamins tested were more attractive than the preferred sugars or amino acids.

Similarly, Lanza et al. (1993) compared the sugar and amino acid preferences of *Solenopsis invicta* and *S. geminate* with the amino acids and sugar components found in extrafloral nectars. Fire ants were fed on pre- and postdefoliation nectars of *Impatiens sultani*, which varied in amino acid content. Both ant species were also fed from artificial nectars designed to mimic those of *Passiflora ambigua*, *P. talamancensis*, and *P. quadrangularis* which varied in their sugar content. Their results (Lanza et al. 1993) indicated that *S. geminate* workers preferred postdefoliation mimics (rich in amino acid content) over the predefoliation mimics (contains relatively low amino acid content). *S. invicta* on the other hand, did not discriminate between the two nectar mimics. In sugar preference tests, both ant species preferred the nectar of *P. ambigua* over *P. talamancensis* and *P. quadrangularis*. It is interesting to note, that the nectar of *P. ambigua*, preferred by both species, contained the lowest total sugar concentration and the lowest calorie content. The preference for *P.ambigua* nectar may indicate that these ants do not necessarily maximize their sugar energy intake when foraging.

Leptothorax and *Monomorium* species were investigated by Lanza and Krauss (1984) with respect to amino acid preferences. Both ant species were offered choices among varying amino acid samples commonly found in both floral and extrafloral nectars. Lanza and Krauss found that both ant species visited sugar solutions prepared with a single amino acid (alanine, arginine, serine, cysteine, methionine or aspartic acid) more frequently than the sugar only controls. *Monomorium* preferred control samples over tyrosine solutions whereas, *Leptothorax* preferred control solutions over histidine solutions. *Leptothorax* did not discriminate between the control and tyrosine solutions. *Monomorium* did not discriminate between the control and histidine solutions. The authors suggest that their results indicate that ants can act as selective agents, by favoring plants with specific amino acids in their nectars.

The initial amino acid preferences tests conducted in this study indicated that *T. sessile* may be able to discriminate among different amino acids. However partial evaporation of the test samples and the small sample size may have generated inconclusive results. Nevertheless, phenylalanine was consumed in greater amounts over other amino acids tested. Phenylalanine is common in foods such as dairy products, meat, bananas, avocados, sesame seeds and pickled herring. It is also found in the sugar substitute, aspartame (commonly known as NutraSweet®). Aspartame is made up of two amino acids, aspartic acid and phenylalanine. Preliminary observations using aspartame and other sugar substitutes such as saccharin indicate that odorous house ants will feed on aspartame, and saccharin to a lesser extent. Still, additional tests using a larger sample size are needed to better define possible amino acid preferences.

Lactalbumin hydrolysate is a common hydrolyzed milk protein that is sometimes called the whey protein. During the making of cheese, lactalbumin is not precipitated out with casein (Ensmingel et. al., 1995). We found no significant differences in the consumption of lactalbumin hydrolysate at varying concentrations from 0.5 – 5%.

Brewer's yeast (from *Saccharomyces*), is a by-product from the brewing industry. It is a rich source of B vitamins, but also contains approximately 35% crude protein

(Ensmingel et. al., 1995). Initial studies that paired brewer's yeast hydrolysate against lactalbumin hydrolysate and casein hydrolysate indicated no significant differences in consumption among the samples.

Lipids are an important component of the diet of many insects. Because odorous house ants will consume lipids to a certain extent, we conducted a series of preference tests on plant and animal derived oils. Oils were found to be generally unacceptable in food preference tests. A significant drop in consumption was observed in sugar samples containing as little as 1% oil.

In lipid preferences tests using menhaden oil, only samples containing a 5% concentration demonstrated a significant drop in consumption. Later experiments showed no significant difference among the samples containing the oil. Menhaden oil is a blend of many different fatty acids. It is a rich source of long-chain Omega 3 polyunsaturated fatty acids and is composed of approximately 15% palmitic acid, 11% oleic acid, 10% palmitoleic acid, 6% myristic and a variety of other lipids that include C18, C20 and C22 fatty acids. It seems that certain fatty acids may be attractive to *T. sessile* but further tests of various fatty acids are needed to identify possible lipid phagostimulants.

It is interesting to note, that in addition to sweet foods, odorous house ants will readily feed on dead insects (**Figure 28**). We do not know which nutrients are attractive to the odorous house ants when foraging on dead insects, or if they exhibit any kind of feeding preferences when feeding on insects. In addition to insects, Clark and Blom (1991) observed *T. sessile* foraging on a dead montane vole, *Microtus montanus* (Peale) in mid April, of 1987. This observation may reflect a need for protein in the spring when queens are returning to egg laying and the colony is rearing brood after an over wintering period of diapause.



Figure 28: Odorous house ants foraging on a dead German cockroach

Though more tests must be conducted to better define specific food preferences of *T. sessile*, it is clear that this ant species is primarily attracted to “sweet” liquid foods containing sucrose. The addition of salts to sucrose samples can generate even greater responses to feeding in odorous house ants. Besides sugars and salts, *T. sessile* is also attracted to hydrolyzed protein containing materials such as casein, and possibly lactalbumin. Additional testing of various fatty acids and other macronutrients will provide a more complete understanding of odorous house ant food preferences. This information should prove useful in the future development of more attractive and effective control programs.

CHAPTER 5 SUMMARY

This research study investigated foraging and food preference behaviors of the odorous house ant (*Tapinoma sessile* (Say)). Results indicated that odorous house ants do not strongly discriminate between time of day or night when foraging. Unlike the black carpenter ant, whose peak foraging activity is strongly defined as nocturnal (Cannon, 1998; Klotz 1984), odorous house ants do not show distinct peaks of activity. When an acceptable food source is encountered, foraging trails are immediately established to the source and foraging continues throughout the day and night until the resource is depleted or is no longer desirable. However, it was observed that *T. sessile* workers have a tendency to forage in greater numbers when temperatures are optimal or needs are greater. In the spring, odorous house ants were not observed foraging at temperatures less than 10 °C. However, in September, most of the foraging activity was observed during the night and early morning when temperatures hovered around 11-14 °C. In addition, temperatures in the summer at some of the test sites were very high and reached above 40 °C with only a slight decrease in foraging activity.

In the laboratory, initial food preference tests showed that carbohydrates, proteins and lipids are all consumed to some degree by foraging odorous house ants. However, when given a choice, carbohydrates (simple sugars) were preferred over proteins or lipids tested. Preferred carbohydrates were found to be sugars commonly occurring in honeydew: sucrose, and to a lesser extent, fructose (Fell and Morse, 1977). Similar sugar preferences were identified for *Camponotus pennsylvanicus*, (Cannon, 1998).

In addition to sugars, hydrolyzed proteins produced positive feeding responses in *T. sessile*. Both casein hydrolysate and lactalbumin hydrolysate were acceptable to foraging ants at a 5% concentration in an agar matrix. The preference for hydrolyzed protein suggests that the ants respond to specific amino acids better than to protein. Initial amino acid preferences tests in this study generated inconclusive results primarily due to the small sample size. Nevertheless, observations indicate that odorous house ants

may be able to discriminate among different amino acids. However, additional studies are needed to better identify specific amino acid preferences in *T. sessile*.

Salts were found to be highly acceptable when added to a 15% sucrose solution. The addition of 0.5% NaCl significantly increased consumption over control samples. Inorganic compounds are essential components of the diet of many insects and it is common to find specific chemoreceptors that respond primarily to inorganic salts (Chapman, 1995). Compounds such as sodium, potassium, calcium, magnesium, chloride and phosphate are essential elements necessary for the proper functioning of cells in insects.

Interestingly, macronutrient collection in odorous house ants did not show seasonality. Tests indicate that odorous house ants will feed continuously on sugary foods through out the spring and summer, as well as on proteins. Although other ant species may indeed demonstrate seasonal preference behaviors for certain types of foods (Tripp et al., 2000), odorous house ants do not. These findings are consistent with Bistows' (1984) research on the benefits of ant attendance to honeydew producing homopteran insects. In Bistow's studies, it was concluded that no clear pattern of seasonal preference for protein or carbohydrate was found for *Tapinoma sessile* or the myrmica species being studied. The odorous house ants and myrmica ants were found to feed on "sugar rewards" throughout the season.

The traditional approach to the control of odorous house ants is the application of a spray formulation insecticide in and around the structures where ants have been observed. However, this method may exacerbate problems by causing a colony to split into multiple units, each of which can become a separate colony. A more effective approach would be to use baits, a technique that can provide several advantages. Colonies do not have to be located for control to be effective, and the amount of active ingredient placed into the environment can be reduced. It is important to continue researching food preferences and foraging behaviors of odorous house ants as well as documenting the behaviors and

habits of other pest ant species. Without a basic knowledge of their biology, the control of ants and other urban pest species will continue to be a challenge for the consumer, home owner and pest management professional alike.

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VITAE

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