AN ABSTRACT OF THE DISSERTATION OF

Mark E. Mankowski for the degree of Doctor of Philosophy in Forest Products presented on December 6, 2001. Title: Biology of the Carpenter Ants *Camponotus vicinus* (Mayr) and *Camponotus modoc* (Wheeler) in Western Oregon.

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Abstract approved: _____
Jeffery J. Morrell

Pest control operators in Oregon were surveyed to examine the occurrence of carpenter ants and other wood destroying organisms in structures. Carpenter ants frequently occurred in the coast and Willamette valley regions of the state and were associated with wetter, mesic habitats.

To examine the effects of substrate and moisture on *Camponotus vicinus* and *Camponotus modoc*, I exposed inseminated queen ants to various substrates at differing humidities and exposed queens ants to a series of humidities. *Camponotus vicinus* intiated significantly more colonies in drier conditions than *C. modoc*. *Camponotus vicinus* also lived longer at lower humidities than *C. modoc*. Neither ant species could rear offspring in western redcedar.

A survey of the yeasts associated with *Camponotus vicinus* revealed the yeast *Debaryomyces polymorphus* was frequently found in the buccal cavity and in colonies of these ants at two different locations. To examine the effects of exposure to this yeast on larval development in *C. vicinus*, I developed an artificial diet for this ant and tested this diet and variations of it on small satellite colonies of ants and larvae. Some of the artificial diets significantly affected larval development.

Exposure of the small colonies and larvae to *D. polymorphus* showed that the yeast effected growth of some colonies fed deficient diets. Ants fed diets lacking B vitamins and cholesterol had heavier pupae and brood when exposed to live yeast than those not exposed.

Biology of the Carpenter Ants *Camponotus vicinus* (Mayr) and *Camponotus modoc* (Wheeler) in Western Oregon.

By
Mark E. Mankowski

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Mark E. Mankowski, Author

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DEDICATION

Dedicated to my father Edward H. Mankowski

1936-1999

Biology of the Carpenter Ants *Camponotus vicinus* (Mayr) and *Camponotus modoc* (Wheeler) in Western Oregon.

CHAPTER 1: INTRODUCTION

1.1: GENERAL INTRODUCTION

Due to their habit of nesting in wood, carpenter ants have long been recognized as pests of wooden structures (Pricer, 1908; Fowler, 1986; Akre, et al. 1995). Originally considered to be a nuisance pest, (Fowler, 1983) carpenter ants have recently been categorized as much more economically damaging (Hansen and Akre, 1985; Akre and Hansen, 1988; Akre et al. 1995). In an extensive study of infestation in Washington State, Hansen and Akre (1985) found that carpenter ants were responsible for at least 42,000 annual structural treatments by pest control operators. Fowler (1986) estimated from several sources in the northeastern U.S. that these ants cause millions of dollars in damage annually.

Aside from damage to structures, carpenter ants also have been observed to cause extensive damage to telephone poles (Friend and Carlson, 1937; Shields, 1996) as well as to shade and ornamental trees (Fowler, 1983). Hansen and Akre (1985) postulated that infestations and loss of merchantable timber by carpenter ants could be a major, yet undocumented problem for many tree species in the northern U.S. and southern Canada.

Carpenter ants belong to the largest genus in the family Formicidae, *Camponotus*. This genus is found worldwide, but has a primarily holoartic distribution with the majority of economically damaging species occurring in nearctic forests (Fowler, 1983). Although they are referred to as carpenter ants, most *Camponotus* species nest in soil, under debris, or in plants and function as efficient predators of forest insects (Akre, et al. 1995). The few species considered pests typically excavate wood or other fibers to create nesting cavities (Akre and

Hansen, 1995). Twenty-three species of *Camponotus* occurring in North America are considered nuisance or structural pests and seven can cause extensive damage to wood and structures (Akre, et al. 1995).

1.2: BIOLOGY OF CARPENTER ANTS

Information on the biology of *Camponotus* exists only for a few species, but is generally presumed to be as follows: mature colonies of *Camponotus* produce both male and female reproductives in the fall and these reproductives emerge in early spring for mating flights (Holldobler, 1961). Mating flights are mediated by mandibular gland secretions from male ants that stimulate females to begin their mating flight. Mated males die and the inseminated females select a nesting site, usually in a small cavity in a stump, log, under bark or in moist timbers of a building (Fowler, 1986; Akre, et al. 1995). The queen then lays anywhere from 10 to 25 eggs that hatch into larvae within two weeks. The queen feeds this first batch of brood from fat reserves in her body and never ventures forth from her claustral chamber (Wilson and Holldobler, 1990). The larvae pupate and emerge as minor workers that forage, excavate, and rear the next brood. Normal development from egg to adult is 45-70 days (Pricer, 1908; Hansen and Akre, 1985). A second period of egg laying occurs in July, which is tended by the first brood. After hatching into larvae, this second brood ceases development and overwinters in this stage. Over the next two years foraging workers gather enough food for larger major workers to be produced. When the colony is 6-10 years old it usually has well over 2,000 workers and begins to produce winged reproductives and satellite colonies (Akre, et al. 1995). These reproductives develop in the autumn and overwinter until spring when they emerge for their mating flight.

Parent colonies overwinter in the nest in a diapause that is independent of temperature and photoperiod. Colonies experience two peaks of seasonal activity. The first occurs from January to June when they break their diapause and the queen

begins her first 7-10 day period of egg laying. Food gathering by foraging workers ensues to support the rapidly growing larvae through June (Akre, et al. 1995). A second peak of activity occurs in late July when the queen lays eggs for another 7 to 10 day period. Foraging is shorter and less intense during this period (Fowler 1986; Akre, et al. 1995). Diapause begins in September and late summer brood overwinter as larvae that complete their life cycle the following spring. Carpenter ant colonies are perennial and may exist for up to 20 years. Colonies are predominately monogynous, meaning only one queen is active in a colony at a given time, however, multiple queen colonies have been observed in *C. modoc* (Wheeler) and are common in *C. vicinus* (Mayr). Colonies of the later species can attain a size of 50,000 to 100,000 workers. *C. modoc* generally attains a size of 50,000 workers and *C. pennsylvanicus*, an eastern species has colonies of up to 15,000 workers.

Carpenter ants that nest in wood do not eat wood, but excavate it. In forests, nests are established in living trees, stumps, and logs. The most common species in the Pacific northwest in structures are *C. modoc* and *C. vicinus* (Hansen and Akre, 1985). Of these two species, *C. modoc* occurred in 75% of structures investigated. These ants prefer wall voids and will mine insulation (Akre, et al. 1995). Although carpenter ants commonly occur in moist wood that is in some state of decay, they also extend their galleries into sound wood surrounding decayed areas (Akre, et al. 1995). As colonies mature, they may form satellite colonies that consist of workers, older larvae, and pupae. These satellite nests are generally found in drier locations than the parent colony. The parent colony, which is usually in a wetter location, contains the queen, eggs, and early instar larvae (Hansen and Akre, 1985; Akre, et al. 1995).

Although the life cycle and biology of some carpenter ant species has been studied extensively, much remains unknown (Fowler, 1986; Akre, et al. 1995). Only a few species of carpenter ants in North America have been studied and the majority of these occur in eastern North America.

The objectives of our studies were to further our knowledge of carpenter ants by surveying the occurrence of these ants and wood destroying organisms in the Oregon and to investigate the biology of two species of carpenter ant *Camponotus modoc* and *Camponotus vicinus* particularly in reference to the ability of these ants to initiate colonies and survive in desiccating conditions. We also investigated the association of microorganisms, particularly yeasts, with *Camponotus vicinus* and examined whether yeasts aided in ant nutrition.

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CHAPTER 2: INCIDENCE OF WOOD-DESTROYING ORGANISMS IN OREGON RESIDENTIAL STRUCTURES.

Mark Mankowski and J.J. Morrell Forest Products Journal Vol:50(1):49-52

2.1: ABSTRACT

The incidence of wood-destroying organisms in homes was surveyed in Oregon. Decay fungi were the most common wood-destroying organisms, except in the drier, eastern region, where termites were more common, and in the wet, coastal region, where wood-boring beetles (Anobiidae) were equally common. A number of building practices appeared to be related to the incidence of wood-destroying organisms, but the most common was the presence of woody debris around and under buildings. The results suggest that better education of builders and homeowners about removal of woody debris would help reduce the incidence of wood-destroying organisms in buildings.

2.2: INTRODUCTION

Wood generally performs well in residential structures that are properly designed, constructed, and maintained (Verrall, 1986; Verrall and Amburgey, 1980). Often, however, one aspect of this triad is neglected, permitting the entry of wood-destroying organisms (WDOs), such as decay fungi and insects (Scheffer, 1991).

The incidence of WDOs in houses is often ignored because it occurs individually, and there are no national reporting systems to collect data on losses caused by these organisms. We therefore must estimate losses, but even estimates provide a staggering glimpse of the effects of WDOs on our economy. For

example, total annual losses from WDOs were estimated to be 2 billion dollars in 1978 (Levi and Moore, 1978). Ten years later, Brier et al. (1988) estimated that WDOs caused 364 million dollars in damage per year in California alone. Su et al. (1991) estimated that subterranean termites accounted for 80 percent of the 1.5 billion dollars spent per year in the United States for termite control. Wood-destroying organisms are most often detected during the inspections that many financial institutions require prior to the sale of a house. In some states, these reports are retained for some period after inspection, either by a state agency or by the pest inspector. These forms could provide a wealth of data regarding associations between building details and infestations and could be used to prioritize research on WDOs and their control. With the exception of the survey in California (Brier et al., 1988), however, these reports do not appear to be used for any of the above purposes. We surveyed pest reports from pest control operators (PCOs) in the state of Oregon to determine the incidence and location of WDOs in residences.

2.3: MATERIALS AND METHODS

The state of Oregon was divided into 5 geographic regions based primarily on mountain ranges and average annual rainfall (Figure 1). A list of members of the Oregon Pest Control Association was used to select PCOs for the survey. This group of companies uses a standard reporting form for WDOs, which simplified our survey and increased the uniformity of the resulting data. Although PCOs look for both decay and insect damage, their training places more emphasis on the detection of insect infestation and damage. On their report forms, PCOs are required to identify WDOs and conditions favorable to infestation or infection and the areas where any structural damage existed.

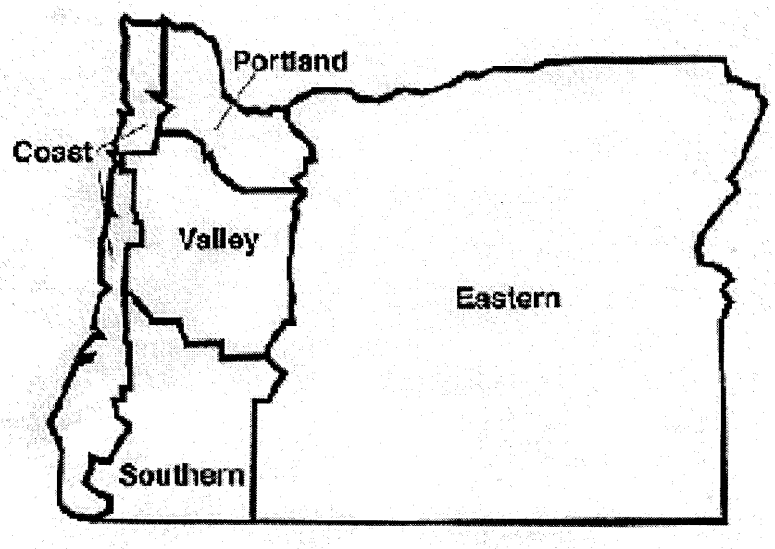

Figure 2.1: Regions of Oregon used to assess the relative frequency of wood-destroying organisms in homes.

Eight PCOs were selected for the survey: two in the Portland, Willamette Valley, and coastal regions and one each in the eastern and southern regions. We chose two PCOs for the Willamette Valley and Portland regions because those regions contain most of the state's population. We chose two PCOs for the coastal region because of the extreme length of the coast (>300 miles). Because the other regions had lower populations, we surveyed only one PCO for each.

At each PCO, we randomly selected a minimum of 50 wood-destroying organism reports from the files generated in 1996 and 1997. The surveys were used to identify county and inspector, as well as the presence and location of fungi, dampwood termites, subterranean termites, carpenter ants, and wood-boring beetles. We noted the type of house (full basement, concrete slab, or crawl space) and the presence of any contributing factors, such as wood debris, soil/wood contact, excessive moisture or plumbing leaks, inadequate ventilation, faulty grade or fill, inadequate clearance, and the presence or absence of vapor or foil barriers

(Appendix). We examined 463 reports and tabulated the data from each house type and region.

2.4: RESULTS AND DISCUSSION

2.4.1: Incidence of wood destroying organisms

Fungi were the most common WDO in four of the five regions (Table 2.1). The exception was eastern Oregon, where subterranean termites were the most abundant WDO. The occurrence of large numbers of subterranean termites in the eastern region was perplexing because this zone has the driest and coolest climate of the five regions. Subterranean termites were a relatively minor pest in the four remaining regions, occurring in 7 to 13 percent of the dwellings examined. Dampwood termites (*Zootermopsis augusticollis* Hagen), a species specific to the Pacific Northwest, were absent from the eastern region and present at low levels in all but the coastal region. The occurrence of higher levels (18%) of this species along the coast follows closely with their requirement for wood at an elevated moisture content. Houses in this region are subjected to wind-driven rain and foggy conditions, which contribute to the elevated moisture levels that support this insect.

Carpenter ants appeared to be slightly more abundant in both the coastal and Willamette Valley regions. These insects generally require moist to wet wood to initiate colony formation, so the increased rainfall levels associated with these two regions may affect the incidence of carpenter ants. Wet wood is softer and easier to tunnel, potentially enabling workers to increase the rate of excavation. Satellite colonies of carpenter ants are less discerning in their moisture requirements and are found in drier wood.

Table 2.1: Incidence of wood-destroying organisms (WDOs) in homes on five regions of Oregon.

		Frequency (% of the number of homes reporting WDOs)				
Region	No. of homes	Fungi	Dampwood termites	Subterranean termites	Carpenter ants	Wood-boring beetles
Coast	92	70	18	11	21	28
Portland	66	44	2	11	9	9
Valley	134	53	2	13	21	4
Southern	109	61	5	7	6	8
Eastern	62	47	0	68	13	5

Wood-boring beetles, notably anobiid powderpost beetles (Anobiidae), were especially prevalent in the coastal region. Anobiids generally require moisture contents between 12 and 20 percent (Suomi and Akre, 1992). The elevated humidity and higher precipitation associated with the coastal region would result in wood closer to the moisture requirements of these beetles. Wood-boring beetles were relatively minor in the other regions, with infestation levels ranging from 4 to 9 percent of the houses inspected.

2.4.2: Relationship between building components and wood-destroying organisms

Wood-destroying organisms were more likely to be associated with specific components of a building. These preferences reflect combinations of proximity to soil and the likelihood that moisture could collect in that area. Decay fungi appeared to be widely distributed among all building components except the roof (Table 2.2). These data must be viewed cautiously, however. Most Oregon PCOs do not actively investigate the roof to the extent that they remove shingles and evaluate the condition of the wood beneath, where decay is most likely to occur. Roof reports likely describe conditions on a relative basis, using only the visible portion of the shingles.

Decay fungi were the dominant organisms in most building components, except for sills/joist/subfloor and interior frames/windows in the eastern region, where subterranean termites were the dominant organisms. In most other cases, insects represented a minor component of WDOs found in homes. Pest control operators in the eastern region may need to be more vigilant concerning subterranean termites, while those on the coast need to be more concerned about wood-boring beetles and dampwood termites.

2.4.3: Factors favoring wood-destroying organisms

Builders have long known how to avoid infestations by WDOs, but translating that knowledge into practice has been difficult. Practices that include avoiding direct contact between soil and wood, eliminating sources of moisture, removing woody debris from crawl spaces, and ensuring that the exterior grade does not channel water toward the house are often ignored, resulting in the development of conditions that favor WDOs (Scheffer and Moses, 1993). Our results concur with Wilcox and Dietz (1998), who found that factors such as type of siding, roof leaks, and insufficient ventilation can make structures more susceptible to wood decay. The length of time between construction and detection of WDOs may sometimes obscure connections that might prescribe changes in design, construction, or maintenance of a structure. However, Wilcox and Dietz (1998) found that the greatest amounts of visible decay occurred in younger (6–10 years old) structures in California.

Table 2.2: Incidence of wood-destroying organisms (WDOs) in various locations of homes in five regions of Oregon.

	Frequency (% of houses within region reporting WDOs)				
Location/region	Fungi	Dampwood termites	Subterranean termites	Carpenter ants	Wood-boring beetles
Sills-joists-subfloor					
Coast	21.7	8.7	6.5	5.4	21.7
Portland	10.6	0.0	6.0	3.0	6.0
Valley	17.9	<1.0	3.7	3.0	<1.0
Southern	32.0	2.7	5.5	1.0	<1.0
Eastern	21.0	0.0	48.4	8.0	3.2
Interior frames/window					
Coast	5.4	1.0	0.0	3.3	3.3
Portland	9.0	0.0	1.5	1.5	1.5
Valley	14.9	0.0	2.2	2.2	0.0
Southern	22.5	0.0	1.8	0.0	0.0
Eastern	14.5	0.0	22.6	3.2	1.6
Exterior Doors/windows					
Coast	26.0	1.0	1.0	1.0	1.0
Portland	12.1	0.0	0.0	1.5	0.0
Valley	13.4	0.0	<1.0	0.0	0.0
Southern	5.5	0.0	0.0	0.0	0.0
Eastern	3.0	0.0	1.6	0.0	0.0
Roof					
Coast	5.0	0.0	0.0	0.0	0.0
Portland	0.0	0.0	0.0	0.0	0.0
Valley	1.5	0.0	0.0	0.0	0.0
Southern	0.0	0.0	0.0	0.0	0.0
Eastern	0.0	0.0	1.6	0.0	0.0
Siding					
Coast	31.5	1.0	0.0	1.0	1.0
Portland	15.0	0.0	0.0	0.0	0.0
Valley	13.4	0.0	0.0	2.2	<1.0
Southern	15.6	0.0	0.0	0.0	0.0
Eastern	14.5	0.0	1.6	0.0	0.0
Porch/deck					
Coast	24.0	2.0	0.0	2.2	1.0
Portland	13.6	0.0	0.0	0.0	0.0
Valley	17.9	0.0	<1.0	0.0	0.0
Southern	9.2	0.0	1.0	1.0	0.0
Eastern	9.7	0.0	3.2	1.6	0.0

The WDO reports surveyed indicated that a high percentage of homes had one or more of the above-mentioned factors, which can provide an environment conducive to WDOs. For example, the incidence of soil/wood contact ranged from 22 to 68 percent of the houses surveyed, the presence of wood debris ranged from 17 to 63 percent, and faulty grades ranged from 7 to 23 percent (Table 2.3). The presence of wood debris and soil/wood contact were especially high in the eastern region and probably made a strong contribution to the extraordinary levels of subterranean termite infestations noted there.

One factor that is often overlooked in structural designs is accessibility for inspections. Homeowners should be able to inspect their structures for the early signs of infestation or infection. This can be facilitated by making the areas most likely to experience infestation accessible, yet 42 to 94 percent of the houses inspected had areas that were defined as inaccessible to the inspector. The preponderance of inaccessible areas sharply increases the likelihood that any infestations that do occur will proceed to a much greater level of damage before they are detected. This increases the costs of repair and can make control more difficult.

Table 2.3: Frequency of specific conditions that favor infestations by wood-destroying organisms in homes inspected in five regions of Oregon.

		Frequency (% of houses in region)								
Region	No. of houses	Wood debris	Soil/wood contact	Excessive moisture	Inadequate ventilation	Faulty grade	Dirt fill	Inadequate clearance	Plumbing leak	Inaccessible areas
Coast	92	34	48	30	10	15	12	25	2	54
Portland	66	24	35	12	14	6	2	5	0	76
Valley	134	41	22	15	5	22	6	5	8	94
Southern	109	17	33	8	5	7	4	16	3	42
Eastern	62	63	68	29	15	23	34	16	5	77

2.4.4: Implications

Although the PCOs report damage caused by fungi, they may overemphasize damage caused by insects because much of their training focuses on the detection and control of these organisms. Although insects are important degraders of wood under specific applications, decay fungi remain the dominant organisms in building deterioration in Oregon. The focus on insect detection has critical implications for preventing deterioration in buildings. At present, there are few remedial treatments, other than drying out the wood, that can be applied to arrest decay in inhabited buildings because of concerns about safety and liability. Oil-based treatments such as copper naphthenate are often used, but they lack the ability to penetrate far beyond the surface and, therefore, have little effect on fungi established more deeply in the wood. Boron is widely used for this purpose, but this chemical cannot penetrate through dry wood and its distribution may not result in complete cessation of fungal attack. The development of alternative methods for arresting decay before substantial damage occurs or more effective methods for detecting decay at the early stages should be substantial goals of those interested in controlling pests in structures.

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CHAPTER 3. SUBSTRATE TYPE AND MOISTURE REQUIREMENTS IN RELATION TO COLONY INITIATION IN TWO CARPENTER ANT SPECIES.

Mark Mankowski and J.J. Morrell

3.1: ABSTRACT

We studied conditions necessary for optimal colony initiation in the carpenter ant species *Camponotus modoc* (Wheeler) and *Camponotus vicinus* (Mayr) on various substrates conditioned to different moisture contents. *Camponotus modoc* and *C. vicinus* queens were placed in Douglas-fir, western redcedar and Styrofoam blocks conditioned in sealed chambers at 70%, 80% or 100% relative humidity. Chambers were periodically monitored for changes in substrate weight, numbers of eggs, larvae, pupae, and worker ants produced. Brood counts produced after 12-13 weeks were used to assess the effects of substrate and moisture content on colony initiation. Queens of *C. vicinus* in Douglas-fir and Styrofoam produced worker numbers that did not differ significantly with moisture content, however, the number of colonies initiated by *C. modoc* differed significantly with moisture content. The results indicate that colony initiation in *C. vicinus* is less sensitive to moisture content than *C. modoc* for Douglas-fir and Styrofoam. No differences were found between moisture contents for ant queens in western redcedar, due to a lack of colony initiation. These results suggest that redcedar was detrimental to the development of early brood in both ant species. In another test, groups of queen ants of each species were exposed to 20%, 50%, 70%, and 100% relative humidity and the time until 50% mortality occurred for each species was recorded. *Camponotus vicinus* lived significantly longer at each of the test humidities than *C. modoc*, suggesting that the former species may be better adapted for xeric environments.

3.2: INTRODUCTION

Carpenter ants are considered nuisance pests in many parts of North America, but are categorized as important structural pests in the northeast and northwestern U.S. Excavation of extensive galleries in wood can seriously compromise the physical properties of wood in service.

Wood in service in the Pacific Northwest is most commonly attacked by *Camponotus modoc* and *Camponotus vicinus* (Hansen and Akre, 1985). Carpenter ants prefer wall voids and will mine insulation (Akre and Hansen, 1995). Although carpenter ants are believed to prefer moist wood that is in some state of decay there has been no research to elucidate whether or not carpenter ants can found colonies in dry wood. It has been observed that established colonies will extend their galleries into sound wood surrounding decayed areas (Akre and Hansen, 1995). As colonies mature, they may form satellite colonies composed of workers, older larvae, and pupae that are generally found in drier locations than the parent colony. The parent colony, which remains in a location of high moisture, contains the queen, eggs, and early instar larvae (Hansen and Akre, 1985; Akre and Hansen, 1995)

Optimal wood moisture or decay levels for colony initiation are unknown (Pricer, 1908; Hansen and Akre, 1985; Fowler, 1986; Akre, et al. 1995). *Camponotus vicinus* is found in both dry and wet regions of the west and it may, therefore, be more tolerant of dry conditions. The ability to initiate colonies in dry wood would be essential for success of *C. vicinus* in drier sites, but the ability of queens of this species to initiate colonies at lower relative humidities such as those found in a structure is unknown.

Conserving water is a constant dilemma for any small terrestrial organism since their size gives them a high surface to volume ratio. Evaporative water loss is related to surface area, and small organisms face a far greater water conservation challenge (Hood and Tschinkel, 1990). Few studies have examined the importance of moisture in ants (Hood and Tschinkel, 1990; North, 1991; Phillips and Jusino-

Atresino 1996; Quinlan and Lighton, 1999) and none have examined the effects of humidity or moisture thresholds in carpenter ants. The excavation of galleries inside wood may enable carpenter ants to maintain more stable moisture levels despite harsh exterior conditions. Although this may be the case, *C. modoc* occurs in more forested areas of the northwest whereas *C. vicinus* is common in more open environments. The presence of *C. vicinus* in more xeric environments implies that it may be able to survive better in drier conditions than *C. modoc*.

Carpenter ant alates leave the colony and mate when the outside relative humidity is high and their exposure to desiccation is low. Dry conditions after a nuptual flight or after initial colony formation may limit colony establishment (Munroe, et al. 1996), but little data exists on the effects of moisture levels on carpenter ant colony initiation. Avoiding conditions suitable for colony initiation could represent one component of strategies for limiting carpenter ant damage in buildings. Thus, we wondered if queen ants of these two species could initiate colonies at low relative humidity in wood and non-wood substrates that were considered too dry for colony initiation or colony survival. We also investigated the effects of relative humidity on survival of queen ants of each species.

3.3: MATERIALS AND METHODS

3.3.1: Colony initiation

Camponotus modoc and *Camponotus vicinus* queens were collected in various parts on Benton Co., Oregon between May and June 1998 and 2000. Queen ants were collected in open paved areas at night when queen ants were searching for nesting sites. Captured queens were temporarily reared in test tubes into which damp paper toweling had been placed. The tubes were incubated in the dark at 25° C.

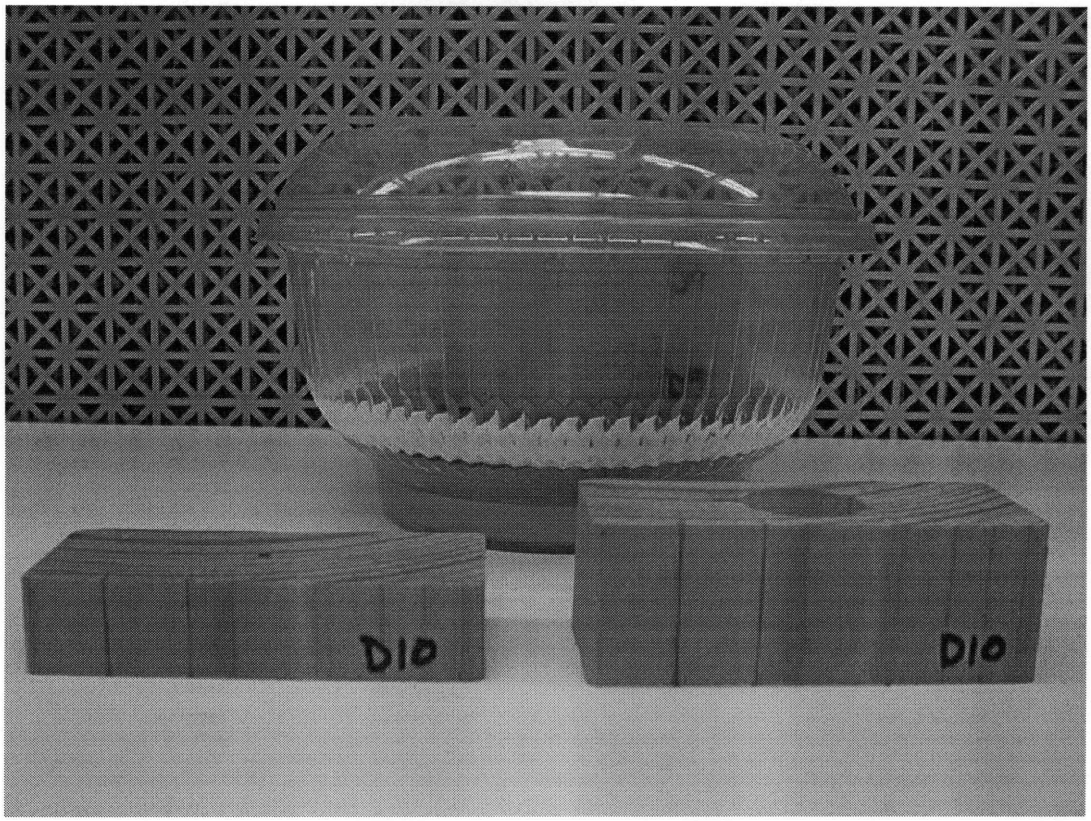

Figure 3.1: Experimental setup for the colony initiation test showing chamber (back) with controlled relative humidity and top (left) and bottom (right) of the blocks used to house queen ants.

Figure 3.1 shows the chamber and substrate blocks used for the experiment. Chambers for housing collected ant queens consisted of plastic food containers with airtight lids. Relative humidity in the chambers was controlled by placing 125 ml of water, potassium chloride, or saturated sodium chloride in the bottom of the container (Winston and Bates, 1960). These solutions maintained relative humidities of 70%, 80%, or 100%, respectively. Thirty-eight blocks, measuring 90x40x50 mm long were cut from Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), western redcedar (*Thuja plicata* Donn ex D. Don), and Styrofoam. Each block was further cut into a bottom section measuring 90x40x32 mm long and a top section measuring 90x40x18 mm long. A 25 mm diameter by 6 mm deep hole was

bored into the top of the bottom section. A 5 mm diameter hole was bored through the top section. The two pieces were then reattached and held in place with a rubber band. Blocks were oven dried at 54° C, weighed and placed into the plastic chambers on waxed cardboard discs over either a salt solution or water to produce the desired relative humidity (RH). Blocks were equilibrated to their respective moisture contents for two weeks prior to the addition of the queens. In 1998 we tested all three substrates at two RH's, while two substrate types at three RH's were tested in 2000. We added a third RH of 80% in 2000 as median RH between the 100 and 70% RH's used in 1998.

The inseminated queens were removed from the test tubes after two weeks and placed, with any eggs they had laid, into the 25 mm diameter by 6 mm deep hole in the bottom section of each wood block. Eggs were handled with a horsehair brush. The top section was then placed on the block to seal the queen ant in her chamber and the block was placed back in the chamber and its lid closed. Table 3.1 represents the number of replicates used for each substrate type for the times that the experiment was conducted. A total of eight *Camponotus modoc* queens and six *Camponotus vicinus* queens were exposed to each substrate type at 70 or 100% RH in 1998.

Table 3.1: Number of queen ants and different humidities used per substrate.

Year		1998		2000		
Humidity		70%	100%	70%	80%	100%
Species	*Camponotus modoc*	4	4	8	8	8
	Camponotus vicinus	3	3	8	8	8

This gave a total of 21 replicates for each relative humidity, substrate, and ant species. Twenty-four *C. modoc* or *C. vicinus* queens were evaluated per

substrate in 2000. Eight replicates per ant species, per substrate, and relative humidity (70, 80, or 100%) were used. A total of thirty-two replicates were evaluated at each relative humidity in 2000. The chambers were incubated at room temperature and monitored every 14 days for changes in wood weight and numbers of eggs, larvae, pupae, and worker ants present. Once the first brood had eclosed, the colonies were supplied with sugar water and chopped insect parts (Hansen and Akre, 1985; Gibson and Scott, 1989). Brood could leave the blocks through the smaller diameter hole in the top section of the block. Final brood counts for the season were made at the end of August after 13 weeks in 1998 and 12 weeks in 2000. This time was selected because colonies produced worker ants around the eight-week point and food and water were placed in the chambers for the workers to consume. Because the addition of food and water four weeks before may have altered the wood EMC at we chose to end the experiment at 12 weeks. The results were used to analyze the effects of wood species and wood moisture content on colony initiation.

3.3.2: Survival at differing humidities

We assessed survival at differing humidities by exposing queen ants of each species to varying relative humidities and determining an LT_{50} for carpenter ant queens exposed. We set up an experiment similar to Hood and Tschinkel, 1990. Mated queens of *C. modoc* and *C.vicinus* were collected as above and placed into tubes containing wet paper toweling until needed. Four queens of each species were individually placed into 12 mm high by 40 mm diameter plastic petri dishes that had between 35 and 40 ventilation holes. The petri plates were in turn placed into chambers over saturated sodium chloride, calcium nitrate, or water to create 50%, 72% or 100% humidity (Winston and Bates, 1960). Another treatment was set up placing petri plates over ceramic molecular sieves, 5A,beads, 4-8 mesh Aldrich®. The sieves were dried at 54° C for 48 hours and placed in the bottoms of

containers to create a humidity of 20%. Three replicates were tested at each of the relative humidities. Relative humidity was verified in each chamber using a battery-operated hygrometer. Queen ants were given no food or water for the duration of the experiment. Brood, if any was produced, were removed at pupal and adult stage. The chambers were kept in the dark at 25° C and monitored daily. If any queens were found dead the date of mortality was recorded. The experiment was conducted for 140 days. Dead ant queens were dried at 40° C for 48 hours to obtain the total dry weight.

3.3.3: Statistical analysis

Colony initiation: data were recorded if one or more adults were produced from queen ants placed in a particular substrate. PROC Genmod was used to analyze the effects of wood species, relative humidity, and ant species on colony initiation (SAS, 2000). Because this was count type data we compared the number of adults produced by queen ants in the various treatments by using a poisson log-linear model. We wanted to keep main effects of substrate, moisture, and ant species in the model and used drop in deviance tests to determine if particular interactions were significant. Any significant interactions were left in the model. We compared means by examining estimated mean ratios for means generated from the final fitted model. Significant differences between compared means were determined by calculating an approximate 95% confidence interval for the estimated ratio of the means. For the survival test, we used PROC Lifetest to perform survival analysis and compute the survival distribution function for each ant species at each relative humidity. This gave an estimate of the lethal time until 50% of the ants died or LT_{50}. Probit analysis at 140 days did not give enough information about survival of the ant species at the lower humidities tested since at that point most of the ants were dead and we could not discern the effects of these moisture levels on the ants. With survival analysis we could look at instantaneous

survival at any point in time for each ant species at each relative humidity tested. This analysis is probably most appropriate because it calculates the probability of mortality at any given time. We used a used a log-rank test to compare the difference in survivorship between ant species at all time points for a given level of humidity.

3.4: RESULTS

3.4.1: Colony initiation 1998

Brood development for single ant queens and eggs exposed in blocks over the 13-week experimental period is shown for Douglas-fir, western redcedar, and Styrofoam in Figures 3.2, 3.3 and 3.4, respectively. Development times for each species from egg to adult ranged from 8 to 10 weeks, supporting previous reports (Hansen, 1985). Although fewer adults were produced at the lower relative humidity by *C. modoc*, the time from egg to adult was the same.

The number of *C. modoc* adult workers produced in Douglas-fir was lower at 70% relative humidity (0.50 workers per queen) than at 100% (3.00 workers per block). The number of *C. vicinus* produced in Douglas-fir was similar at both relative humidities, although this species produced more adults (3.67) in the lower, 70%, humidity than at 100% where it produced an average of 2.00 workers per queen. Both ant species produced more eggs after 7-10 weeks at the 100% relative humidity, but egg production was greater with *C. vicinus* at this relative humidity (Figure 3.2). This agrees in part with Hansen (1985) who found that a second period of egg laying commonly occurs in mid-summer.

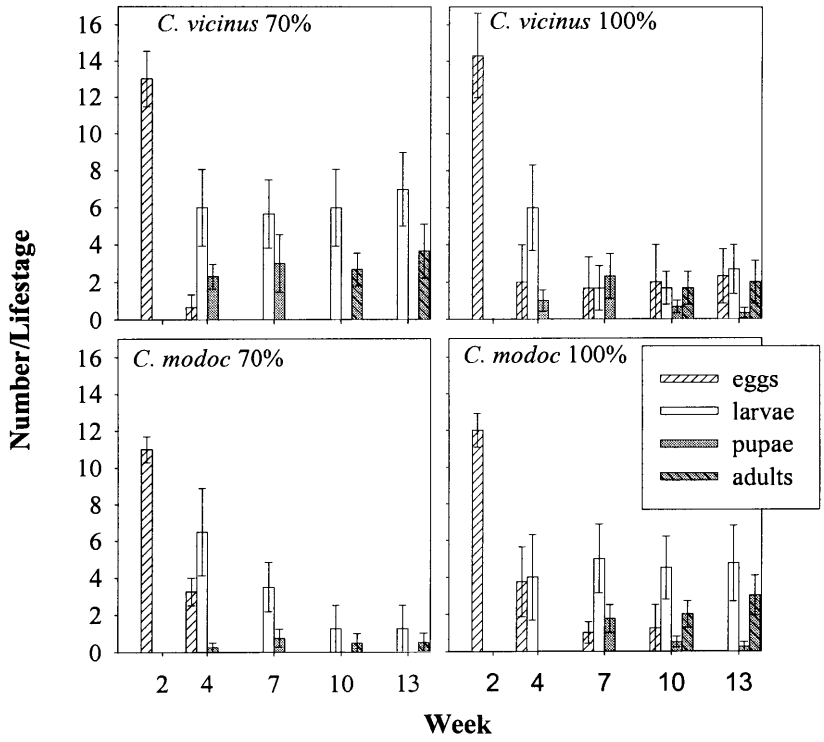

Figure 3.2: Development of two carpenter ant species incubated in Douglas-fir blocks as single queens and exposed at 70% and 100% relative humidity over 13 weeks.

Brood development in western redcedar (figure 3.3) was severely hindered, especially at the higher relative humidity. Both ant species developed to the pupal stage at the lower relative humidity in cedar but no adults were produced. Although *C. vicinus* queens laid more eggs at the 7 and 10-week points, the eggs failed to develop. Eggs placed or laid in the cedar blocks at the high humidity shriveled up and turned brown after 5 to 7 days. Some queens were also observed removing eggs from the blocks at the high relative humidity suggesting cedar blocks at this moisture content were not a preferable substrate for colony initiation and acted as a deterrent to ant development.

Development in the Styrofoam blocks followed trends similar to those

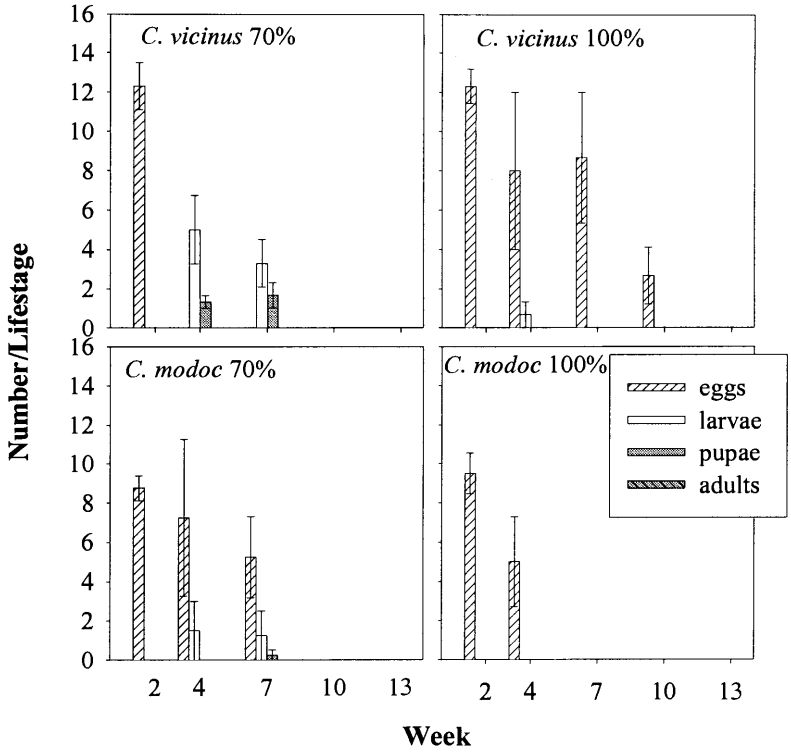

Figure 3.3: Development of two carpenter ant species incubated in Western redcedar blocks as single queens in and exposed at 70% and 100% humidity over 13 weeks.

found in Douglas-fir for *C. modoc*. *Camponotus modoc* produced no workers at the lower, 70%, relative humidity compared to the higher relative humidity where it produced an average of 3.5 workers. Unlike *C. vicinus* in Douglas-fir the *C. vicinus* queens in the Styrofoam substrate produced fewer workers (1.67) at the lower relative humidity than at the high relative humidity where they produced an average of 5.67 workers. However these differences were not as large as *C. modoc* (figure 3.4). These results suggest that *C. vicinus* may be less sensitive to substrate moisture than *C. modoc* in Douglas-fir and Styrofoam.

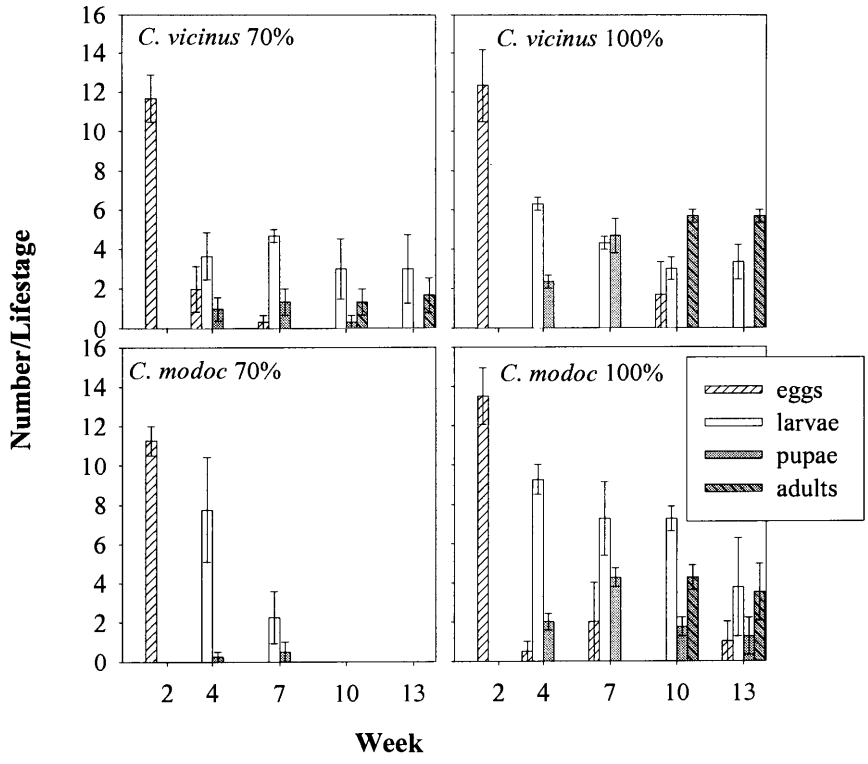

Figure 3.4: Development of two carpenter ant species incubated in styrofoam blocks as single queens and exposed at 70% and 100% relative humidity over 13 weeks.

Figure 3.5 shows the number of worker ants produced by each ant species at the two relative humidities for Douglas-fir, Western redcedar, and Styrofoam sustrates at the end of 13 weeks. The number of workers produced by *C. vicinus* queens in Douglas-fir was 2.00 and 3.67 for 100%, and 70% RH, respectively (Figure 3.5A). *Camponotus modoc* exposed to Douglas-fir appeared to do more poorly at 70% than 100% relative humidity, producing 3.00 and 0.51 workers per block, respectively (Figure 3.5A). No colonies were initiated on western redcedar regardless of relative humidity. *Camponotus vicinus* housed in Styrofoam

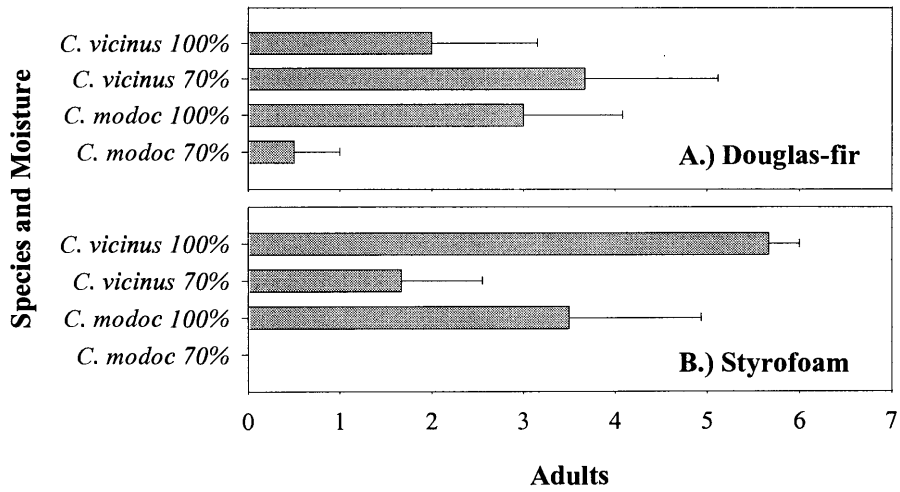

Figure 3.5: Number of adult ants produced for two species of carpenter ant placed as single queens in A.) Douglas-fir and B.)Styrofoam and exposed at 70% and 100% relative humidity in 1998.

produced 5.67 and 1.67 adults per queen in blocks with EMCs of 7.9% and 3.7%, when exposed to 100% and 70% RH, respectively. *Camponotus modoc* reared 3.5 and no workers per queen at 100% and 70% RH, respectively (Figure 3.4B).

Equilibrium moisture content ranges for each ant species exposed to the 100% relative humidity were 21.93-25.33%, 20.13-21.65% and 7.9-8.0% for Douglas-fir, redcedar, and Styrofoam, respectively. For ant queens placed in blocks exposed to 70% RH the EMC ranges were 11.17-11.38%, 9.8-9.87%, and 0.80-3.7% for the same three respective substrates. The EMCs for styrofoam are misleading because this non-wood substrate does not sorb moisture like wood. Colony initiation in *C. vicinus* appeared to be less affected by humidity than *C. modoc* in Douglas-fir and Styrofoam. Western redcedar was detrimental to the development of colonies in both *C. modoc* and *C. vicinus*.

Western redcedar had a drastic affect on the ability of carpenter ants of either species to form colonies. Kruskal-Wallis ranked means for Western redcedar, Douglas-fir, and Styrofoam were 19.5, 43.31, and 46.69 respectively with

a Pr>Chi < .0001 indicating there was a significant effect of substrate. We were concerned, however, that keeping cedar in our model would skew maximum likelihood estimates for the means obtained for the other substrates. We also did not perform the second year study with cedar so for comparative purposes we elected to omit cedar from the final model. With western redcedar removed from the model, we obtained Kruskal-Wallis means of 22.81 and 26.19 for Douglas-fir and Styrofoam, respectively, with a Pr>Chi= 0.40 indicating that there was no species effect on number of adults for these two substrates. We used a Poisson regression model to examine the effects of wood species, relative humidity, and ant species on the number of adult ants produced.

We tested for over dispersion and found the data to be over-dispersed. We used a Prob-F test to test the three way and two-way interactions. The three-way interaction test was not significant (p= 0.644), but a significant interaction existed between relative humidity and ant species (p= 0.02). Substrate had no significant effect on the number of adult ants produced when cedar was eliminated from the model. Means for both substrate types were combined and tested further to see if any significant differences existed between the number of adult ants produced by

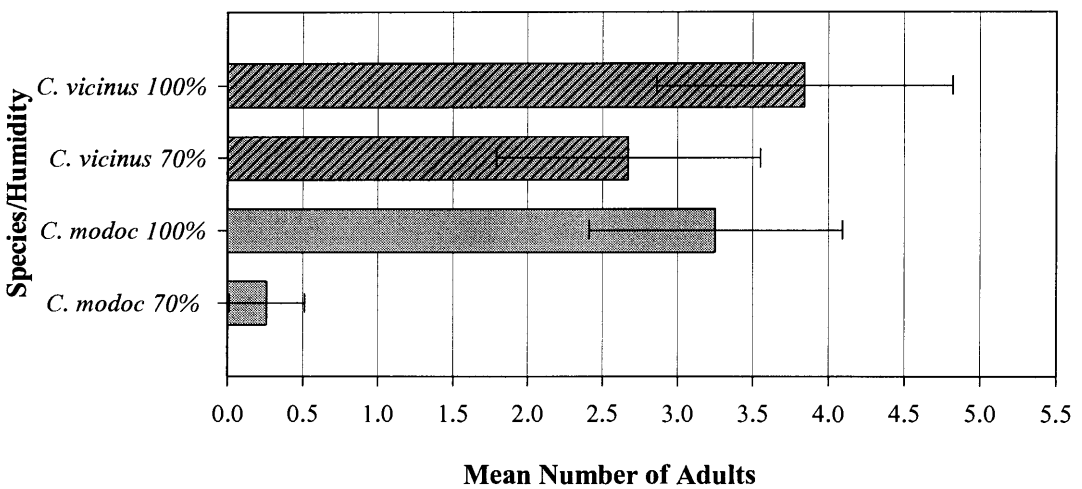

Figure 3.6: Adult ants produced per block by single queens exposed at 70% and 100% relative humidity in 1998.

either ant species at either humidity. Figure 3.6 shows the number of ants produced by queens in 1998 exposed to 70% and 100% relative humidity.

The ratio of means for the mean number of adult ants produced by *C. vicinus* at 100% (3.84) and 70%(2.67) was 1.43 (C.I. 0.60, 3.42). Indicating that the mean number of *C. vicinus* adults produced at 100% was 1.43 times larger than the number produced at 70% and no significant differences existed between those two means. The ratio of means for the mean number *C. modoc* adults at 100% relative humidity (3.25) and 70% (0.26) was 12.05 (C.I. 1.83, 85.63) indicating that *C. modoc* at 100% RH produced 12.05 times more adults than at 70% and the difference between the two means was significant.

The ratio of means for *C. vicinus* adults (3.84) and *C. modoc* (3.25) at 100% was1.82 (C.I. 0.55,2.53), indicating that *C. vicinus* produced 1.82 times more adults at this humidity, but the means did not differ significantly. The ratio of the means for *C. vicinus* (2.67) and *C. modoc* (0.26) at 70% RH was 10.0 (C.I. 1.44, 73.86), showing that *C. vicinus* produced ten times more adults at this humidity and the means differed significantly.

3.4.2: Colony initiation 2000

In the repeated test, *C. vicinus* produced more workers in Douglas-fir than *C. modoc* at all three relative humidities (Figure 3.7). *C. vicinus* produced 1.38, 2.25, and 2.38 adults/queen at 70%, 80% and 100% relative humidity, respectively, while *C. modoc* produced 0.26, 0.76, and 2.13 adults at the same RH's. Of the sixteen *C. modoc* queens tested at 70% RH, one produced two workers. Only *C. modoc* exposed to 100% relative humidity laid eggs at the 7-week point. Development of each ant species in Styrofoam was slightly different in that both species produced the most workers at the 80% relative humidity. *Camponotus vicinus* reared 2.13, 2.75, and 2.13 adults per queen at 70%, 80%, and 100% RH,

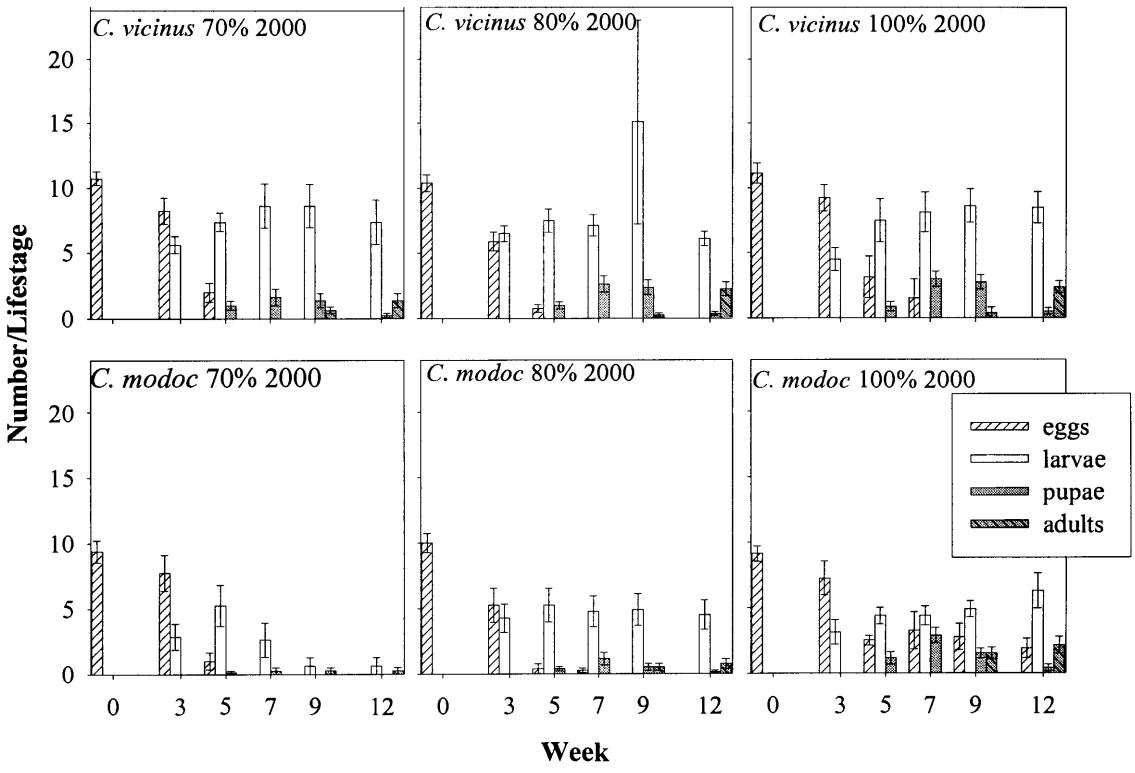

Figure 3.7: Development of two carpenter ant species placed as single queens in Douglas-fir and exposed at 70%, 80%, or 100% relative humidity over 12 weeks in 2000.

respectively, while *C. modoc* produced 0.0, 2.38, and 1.13 adults at the same RH's. An RH of 80% appeared to be optimal for development of both ant species in styrofoam. *Camponotus modoc* laid eggs on this substrate at the 7-week point at 100% RH (Figure 3.8).

Figure 3.9 shows the number of worker ants produced by each ant species at the three relative humidities for Douglas-fir and Styrofoam and the equilibrium moisture contents for each material. The number of workers produced per *C. vicinus* queens in Douglas-fir appeared to be similar for all three relative humidities although fewer ants were produced at 70% RH. Mean number of workers reared was 2.38, 2.25 and 1.38 for 100%, 80%, and 70% RH, respectively. *Camponotus modoc* exposed to Douglas-fir appeared to do more poorly at 70% and 80% RH

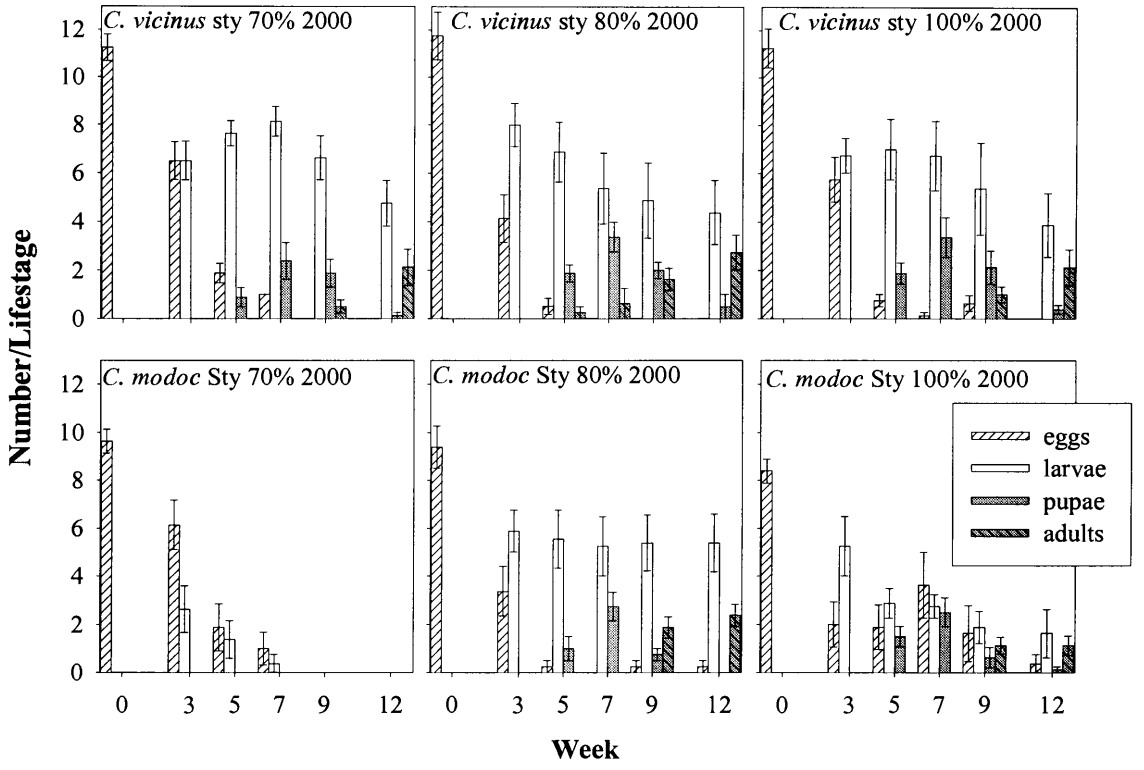

Figure 3.8: Development of two carpenter ant species placed as single queens in Styrofoam and exposed at 70%, 80%, or 100% relative humidity over 12 weeks in 2000.

than 100% RH. *Camponotus modoc* produced 2.13, 0.75 and 0.26 workers per queen at 100%, 80%, and 70% RH, respectively.

Camponotus vicinus produced 2.13, 2.75, and 2.13 ants per queen in styrofoam blocks at the three respective humidities. *Camponotus modoc* produced 1.13, 2.38, and no workers per queen at 100%, 80%, and 70% RH, respectively. *Camponotus modoc* appeared to be affected by lower humidity.

Equilibrium moisture content ranges for either ant species in Douglas-fir were 22.5-23.1%, 16.67-17.35%, and 11.67-11.72% for blocks exposed to 100,80, and 70% relative humidities, respectively. For ants placed in Styrofoam blocks the EMC ranges were 5.23-6.11%, 5.22-6.22% and 0.61-3.47% for the same respective substrates.

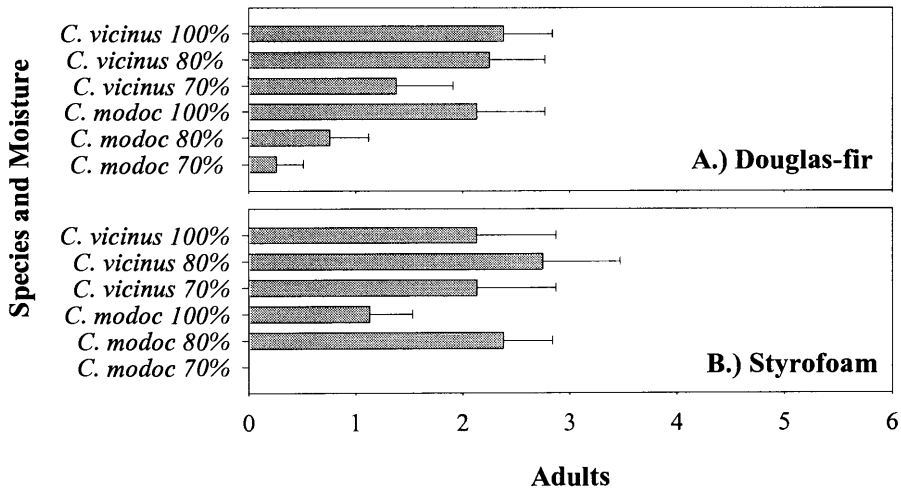

Figure 3.9: Number of worker ants produced for two species of carpenter ant placed as single queens in A.) Douglas-fir and B.) Styrofoam and exposed at 70%, 80%, and 100% relative humidity for 12 weeks in 2000.

As in 1998, we found no three-way interactions and a significant interaction between relative humidity and ant species (p=.012). Data for both substrates were combined (Figure 3.10). *Camponotus vicinus* produced 2.25, 2.5, and 1.75 adults per queen in blocks at 100%, 80% and 70% RH, respectively, while *C. modoc* reared 1.67, 1.57, and 0.13 workers per queen in blocks at the same RH's.

The ratio of means for the mean number of adult ants produced by *C. vicinus* at 100% RH (2.25) and 80% RH (2.50) was 1.11 (C.I. 0.64, 1.93). Indicating that the mean number of *C. vicinus* adults produced at 100% RH was 1.11 times larger than that produced at 80% RH. These differences were not significant. The ratio of means for the mean number of adult ants produced by *C. vicinus* at 80% RH (2.50) and 70% RH (1.75) was 1.43 (C.I. 0.79, 2.59) indicating that the mean number of *C. vicinus* adults produced at 80% was 1.43 times larger than the number produced at 70%, but the differences were not significant. The ratio of the means for the mean number of adult ants produced by *C. vicinus* at 100% RH (2.25) and 70% RH (1.75) was 1.28 (C.I. 0.42, 1.43), indicating that the

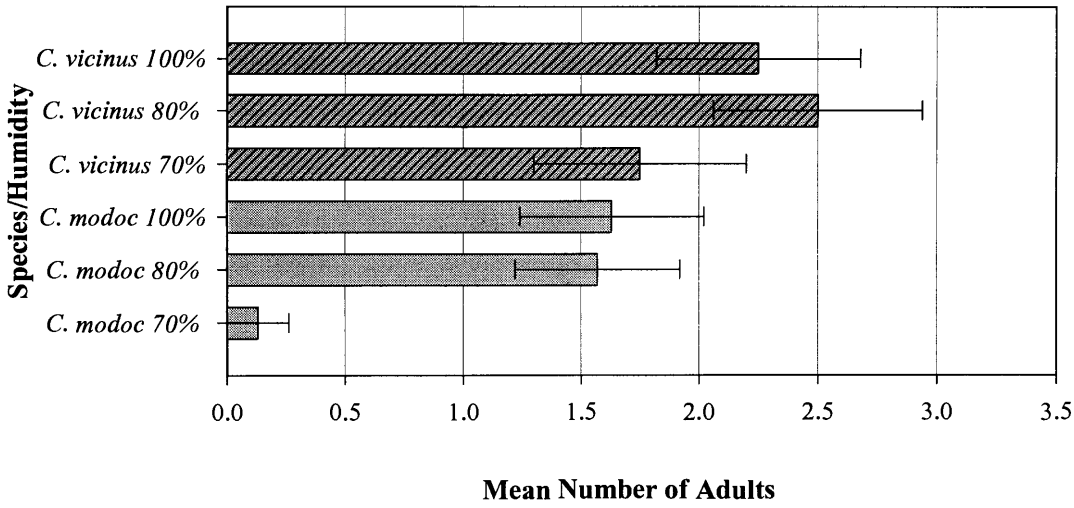

Figure 3.10: Adult ants produced per block by queens exposed at 70%, 80%, and 100% relative humidity in 2000.

mean number of *C. vicinus* adults produced at 100% was 1.28 times larger than the number produced at 70%, but the differences were not significant.

The ratio of the means for the mean number *C. modoc* adults at 100% RH (1.67) and 80% RH (1.57) was 0.96 (C.I. 0.49, 1.89) indicating that *C. modoc* at 100% RH produced 0.96 times more adults than at 80% RH. These results did not differ significantly. The ratio of means for the mean number *C. modoc* adults at 80% RH (1.57) and 70% RH (0.13) was 11.65 (C.I. 2.09, 64.72), indicating that *C. modoc* at 80% RH produced 11.65 times more adults than at 70% RH. These differences were significant. The ratio of means for the mean number *C. modoc* adults at 100% RH (1.67) and 70% RH (0.13) was 12.11 (C.I. 2.18, 61.56), indicating that *C. modoc* at 100% RH produced 12.11 times more adults than at 70% RH and the means differed significantly.

The ratio of means for *C. vicinus* (2.25) and *C. modoc* (1.67) adults produced at 100% RH was 1.38 (C.I. 0.74, 2.58), indicating that *C. vicinus* produced 1.38 times more adults at this humidity, but the means did not differ significantly. The ratio of means for *C. vicinus* (2.50) and *C. modoc* adults

produced at 80% RH (1.57) was 1.6 (C.I. 0.86, 2.96), indicating that *C. vicinus* produced 1.6 times more adults at this humidity, but the means did not significantly differ. The ratio of means for *C. vicinus* (1.75) and *C. modoc* adults produced at 70% RH (0.13) was 13.05 (C.I. 2.36, 72.1), showing that *C. vicinus* produced 13.05 more adults at this humidity and the means differed significantly.

3.4.3:Survival:

Results of the survival analysis indicate that *C. vicinus* out lived *C. modoc* at the four humidities tested. This was particularly evident for *C. vicinus* at the lower, 20% and 50% humidities where this species lived much longer than *C. modoc* (Figure 3.11).

At 20% RH we obtained LT_{50}s of 48.75 days (CI=39.5-57.0) and 14.0 days (CI= 11.5-15.5) for *C. vicinus*, and *C. modoc*, respectively. Results of the log-rank test showed that there was strong evidence $p<0.0001$ that *C. vicinus* lives longer at this humidity than *C. modoc*. At 50% RH, LT_{50}s were 107.75 days (CI=104.5-no upper) and 31.25 days (CI= 25.0-40.0) for *C. vicinus*, and *C. modoc*, respectively. We obtained a p-value of 0.0001 from the log-rank test indicating that *C. vicinus* lived significantly longer at this RH. Interestingly we observed that some *C. vicinus* queens at this relative humidity laid eggs and reared adult brood. This was not the case with *C. modoc* that produced no brood at this humidity.

We obtained LT_{50}s for *C. modoc* of 42.5 days (CI= 38.0-64.0) and 122.0 days (CI= 115-no upper) for 72% and 100% humidities, respectively. *Camponotus vicinus* did not reach 50% mortality at these two higher humidities (Figure 3.11). Results of the log-rank test to compare survival between the two ant species at 72% and 100% humidities were $p<0.0001$ and $p<0.01$, respectively, indicating that *C. vicinus* lived significantly longer than *C. modoc*.

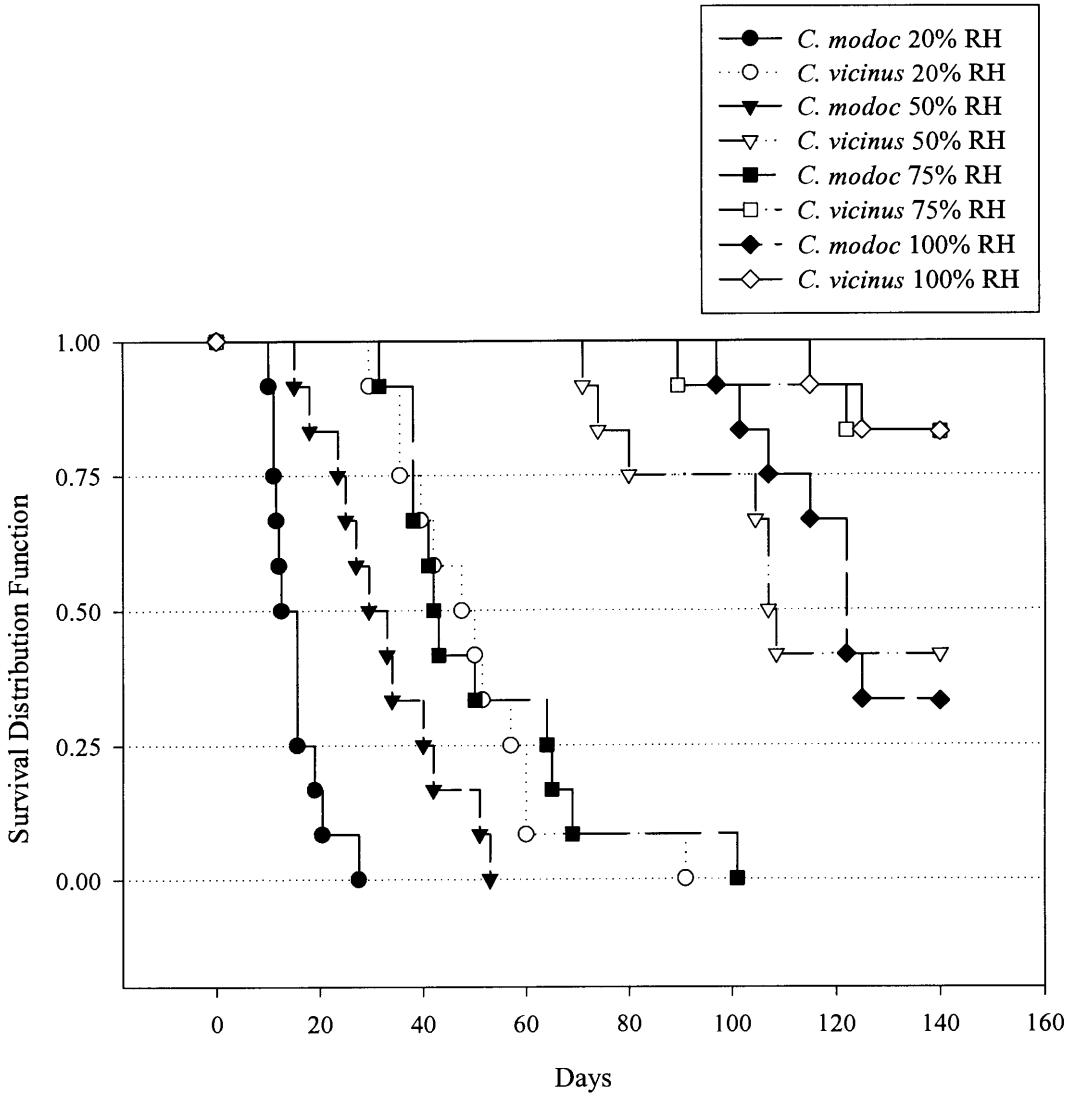

Figure 3.11: Survival response for two species of carpenter ants exposed to four different relative humidities for 140 days.

3.5: Discussion

It is likely that a variety factors dictate where a female carpenter ant eventually founds a colony. Because ants are prone to rapid desiccation because of

their small size, and to predation as long as they are exposed, queens must find nesting places relatively rapidly. Our results strongly suggest that *C. modoc* queens are more sensitive than *C. vicinus* to RH's of 70% or lower. *Camponotus vicinus* queens appear to be relatively unaffected at 70% RH when rearing brood. *C. vicinus* is associated with mesic to xeric habitats in western North America and probably has some ability to conserve water either via respiration or its quantity of cuticular lipids (Hood and Tschinkel, 1990; Lighton and Quinlan, 1994). *Camponotus modoc* is more commonly associated with forest edges and areas of higher rainfall and is probably not as adapted to moisture stress as *C. vicinus*. However, the ranges of these two ants overlap in the Willamette Valley of western Oregon. Our results show that carpenter ants, particularly *Camponotus vicinus*, can initiate colonies in relatively dry wood. This finding contradicts common knowledge that carpenter ants only found colonies in moist wood and suggests that colonies can begin virtually anywhere in a structure. *Camponotus vicinus* is also polygynous and capable of producing large colonies. The ability of this species to resist desiccation makes *C. vicinus* a formidable pest in this region.

Our first year results also suggest that some wood species, particularly, western redcedar, may affect colony growth in carpenter ants. Carpenter ants are known to attack western redcedar utility poles and heartwood in a natural setting. However our results show that this species killed ant brood. It is interesting to note that *C. vicinus* queens reared brood to the pupal stage under drier conditions (70% RH). Drier conditions may have favored development because fewer natural wood extractives in the heartwood could volatize or solubilize and reach the eggs and larvae. The inability of queens to successfully rear workers in redcedar also suggests that carpenter ant colonies in redcedar poles and trees may be by satellite colonies that do not consist of a queen and her young brood or that natural conditions somehow do not affect young brood as they did in our experiment. Western redcedar may be tolerated by older worker castes or aged cedar heartwood may contain fewer materials that limit occupation by carpenter ants. Clearly,

further studies are suggested to determine the nature of the cedar effect and its implications on wood protection.

Exposing inseminated queens to four different humidities indicated that *C. vicinus* can survive for longer periods at lower relative humidity than *C. modoc*. *Camponotus vicinus* had an LT_{50} of 107.75 days at 50% RH. This is longer than the time required for development of eggs to adults for this species. Four of the twelve queens exposed at this RH were able to produce adult workers, suggesting that *C. vicinus* can found colonies at even lower RH than we found in our initial studies.

A similar study exposed arboreal and terrestrial ant species to a similar series of humidities and found that arboreal ants were more tolerant of dry conditions (Hood and Tschinkel, 1990). Although their results showed a correlation with body size and desiccation, they also found evidence that epicuticular lipids and greater rectal pad volume enabled arboreal ants to better cope with desiccation. Further studies are recommended to determine what physiological attributes enable *C. vicinus* to resist desiccation better than *C. modoc*.

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CHAPTER 4: YEAST ASSOCIATES OF THE CARPENTER ANT *CAMPONOTUS VICINUS*

Mark Mankowski and J.J. Morrell

4.1: ABSTRACT

Scanning electron microscopy indicated that the infrabuccal pockets of the carpenter ant (*Camponotus vicinus* Mayr) contained numerous yeast-like cells. Possible yeast-ant associations were examined in six colonies of carpenter ants from two locations in Benton County in western Oregon. Samples from the infrabuccal pocket contents and exoskeleton of worker ants, interior galleries of each colony, and detritus and soil around the colonies were plated on acidified yeast-extract/malt-extract agar and incubated at 25° C. Yeasts were identified on the basis of morphological characteristics and physiological attributes using the BIOLOG® microbial identification system. Yeast populations from carpenter ant nest material and material surrounding the nest differed from those obtained from the infrabuccal pocket. *Debaryomyces polymorphus* was isolated more often from the infrabuccal pocket than from other material. This species has also been isolated from other ant species, but its role in colony nutrition is unknown.

4.2: INTRODUCTION

Although carpenter ants are important structural pests in the northern hemisphere, little is known about their relationships with microorganisms, particularly those found in their digestive tracts. Many insects, particularly xylophageous insects, have associated bacteria, fungi, or protozoa that provide

various nitrogenous compounds and essential vitamins to their hosts (Gusteleva, 1975). Although carpenter ants are not xylophageous, all *Camponotus* species harbor bacterial associates in their mid-intestines (Buchner, 1965).

The digestive biology of the carpenter ant is of interest because a filtering device anterior to the crop, the infrabuccal pocket, prevents worker ants from ingesting solid food particles (Eisner and Happ, 1962). The infrabuccal pocket, a spheroidal pouch found on the ventral portion of the buccal tube (Forbes, 1938), keeps the crop free of particulate matter greater than 150 μm in diameter (Eisner and Happ, 1962). The carpenter ants thus obtain their nutrition from a liquid diet. Because the brood is fed on regurgitated food, the entire protein requirements of a colony must to be supplied by hemolymph and water-soluble proteins that the ants obtain from live and dead insect prey (Ayre, 1963). Carpenter ants obtain carbohydrates from aphid honey dew and from dead and dried insects, but during periods of brood production in early spring and mid-summer, they feed predominantly on insect proteins.

Although the microbial communities of the alimentary tracts of some insects have been studied in great detail, almost nothing is known about the microorganisms living in ants. Only recently have investigators looked at the microbial communities in carpenter ants. Hansen, et al. (1999) found gram negative bacteria and several unidentified yeast species associated with the infrabuccal pocket of carpenter ants. The roles of these organisms are unknown; they may have some digestive function (Hansen, et al. 1999). It may be that carpenter ants obtain free amino acids or essential vitamins from associated microorganisms. In addition, microorganisms in the infrabuccal chamber may be active in the extracellular digestion of large particulate matter that cannot otherwise pass into the ant midgut. Previous studies have found particular species of yeast in the nest material of the forest ant *Formica rufa*; and suggested that these yeasts might play a role in colony nutrition (Golubev and Bab'eva, 1972a, Smith, 1944).

Few studies have examined the fungal associates of *Camponotus*, and none have examined yeasts. Ayre (1963) found large amounts of crop amylase in the

midgut of carpenter ants and concluded that consumption of fungal hyphae might account for the presence of this enzyme. However, no research has surveyed the yeast flora associated with these ants. Yeasts of the genus *Debaryomyces* have been isolated from the nests of *Formica rufa* in Russia (Golubev and Bab'eva, 1972a, 1972b). This yeast was found in great numbers only in active, habitable ant mounds, and this led the investigators to believe that there might be some relationship between the yeast and the ants since the yeasts appeared to produce no adverse effects. However, the actual relationship, if any, was never examined (Golubev and Bab'eva, 1972a, 1972b). More recent investigations of the yeast flora of the fire ant *Solenopsis invicta* suggested that certain yeasts were prevalent in colonies and in larvae of this species (Ba, et al. 2000, Ba and Phillips,1996). Colonies containing certain yeasts also appeared to be more vigorous than those without.

Developing a better understanding of the associations between carpenter ants and microorganisms may help elucidate the role of *Camponotus* in forest ecosystems and may lead to the development of improved control strategies. In a preliminary investigation, we used scanning electron microscopy (SEM) to examine the contents of the infrabuccal pockets of carpenter ants and found reticulated cells similar to those identified by Golubev and Bab'eva (1972a) as *Debaryomyces polymorphus*. This finding led us to wonder whether the same or similar yeast species were associated with *Camponotus vicinus*. We therefore examined yeasts associated with *C. vicinus* over one year in two locations in western Oregon.

4.3: MATERIALS AND METHODS

4.3.1: Microscopy

The contents of the infrabuccal pockets of six *Camponotus vicinus* workers from one colony in MacDonald-Dunn Research Forest near Corvallis, Oregon, were examined by SEM. Ant heads were removed, and the dorsal portion of each head was dissected to expose the infrabuccal chamber. The dissected heads were prefixed for 1 hour at room temperature in a solution containing 0.1M cacodylate (pH 7.3), 2% paraformaldehyde, 2.5% glutaraldehyde, 1% sucrose, and 0.16M $CaCl_2$ and then dehydrated in a graded ethanol series (Nesson, Personal communitcation). The ant heads were dried by critical point drying, and the exposed infrabuccal chambers were ruptured for observation of microorganisms. The six specimens were sputter-coated with gold palladium and examined by using an AMR 1000A SEM (accelerating voltage 20kV, tilt angle 30°, working distance 12 mm).

4.3.2: Yeast isolation from colonies and workers.

Six *Camponotus vicinus* colonies located within 30 km of Corvallis, Oregon, were sampled in January, July, and October 2000 for the presence of yeasts. We sampled colonies from two locations, one in McDonald-Dunn Research Forest and the other from a farm 16 km west of Philomath. Ant colonies were found in logs and under detritus in open areas in both locations. The infrabuccal pockets of 10 medium-sized workers from each colony were sampled after the ants had been collected and washed in sterile PBS solution. The ants were first placed

in a sterile test tube with 10 mL of sterile PBS; the tube was shaken for 1 min with the ants in the solution and then diluted 5x with phosphate buffer solution (PBS) (Hansen et al. 1999).

Twenty-five μL of this extract was plated on yeast-extract/malt-extract agar augmented with 1M hydrochloric acid (7 mL/L; pH=3.7), one plate per dilution, and incubated at 25–27 ° C and examined every 2 days over a 14-day period for evidence of microbial growth. This procedure was performed for three groups of 10 ants per colony.

Infrabuccal cavity contents were obtained by removing the heads of the 10 PBS-washed ants and placing them in 0.05% hypochlorite solution for 60 sec to sterilize the exoskeleton. Surface-sterilized heads were dissected under a compound microscope in a laminar flow hood to minimize contamination. The contents of the 10 excised infrabuccal chambers were placed into 190 μL of buffered sodium phosphate solution. The solution was homogenized in a vortex mixer, and a 6x dilution was performed by placing 50-μL aliquots from the 200-μL dilution onto six plates of the aforementioned medium; observations were made as above.

The interior galleries of each colony were opened using flame sterilized chisels. These implements were frequently resterilized with a bunson burner that was brought to the collection site. One 5-g sample of nest material (substrate) was removed from each colony with sterile chisels and forceps. One sample of frass was collected from under or next to each colony, along with a sample of soil (2–3 g) taken 1.0 m from each nest. Soil was collected by with a sterile chisel that was used as a scoop to remove the upper 50 mm of soil collected. Substrate, frass, and soil samples were placed into pre-sterilized glass petri plates and brought to the lab. The 5-g nest material sample was placed into 10 mL of sterile PBS solution and shaken on a Kleeco® kinetic pulverizer for 30 sec. The resulting solution was diluted 8x, and 25-μL aliquots were spread on plates of the acid medium and observed as above. The 2- to 3-g samples of frass or soil were mixed in 10 mL of sterile PBS for 5 min (Golubev and Bab'eva, 1972a). The suspension was diluted

8x with sterile buffer solution, and 50-µL aliquots were plated on the acid medium (Ba and Phillips, 1996). For all samples, the plates were incubated at 25–27 ° C and observed at 2-day intervals for evidence of microbial growth.

4.3.3: Yeast identification.

Yeasts were identified on the based on physiological attributes by using the BIOLOG® microbial identification system (Biolog, Inc., Hayward, California). The Biolog YT microplate® contains 96 wells to provide 94 biochemical tests to identify the yeast by its' metabolic pattern. Each well tests the organisms' ability to assimilate or oxidize a carbon source. The redox dye tertrazolium violet is used in some wells to calormetrically indicate carbon source oxidation. Assimilation in other wells is indicated by an increase in turbidity (Biolog, 1999). Wells start out colorless but if the compound in the well is assimilated and there is an increase in respiration the cells reduce the tetrazolium dye producing a purple color or increased cell growth increases the turbidity of the suspension in the well (Biolog, 1999).

Unknown yeast isolates were grown on Biolog Universal Yeast® agar for 48 h at 26 ° C, streaked with a sterile swab, and suspended in 12 mL of sterile water. The turbidity of the solution was adjusted to 47% transmittance by using a spectrophotometer. Each unknown yeast was placed in suspension, and three Biolog YT microplates ® were inoculated with 100µl of the suspension and incubated at 26 ° C; they were checked on Biolog's Microlog ® software at 24-, 48-, and 72-h. The time intervals allow a particular metabolic pattern to form that is then interpreted by the Microlog® software.

Yeast isolates from all sampled sites were compared to determine whether

any species were consistently found at all sites and locations. We also wanted to see whether any of the identified yeasts we had found were associated with species of *Formica* and *Solenopsis*.

4.4: RESULTS

4.4.1: Microscopy.

Bacteria and yeast cells were abundant in the infrabuccal pocket of carpenter ants (Figure 4.1). Cells with exterior reticulations similar to those found by Golubev and Bab'eva (1972b), but with smaller dimensions than those found in nests of *Formica rufa*, were prevalent in the infrabuccal pockets. Microorganisms were not restricted to any one area of the infrabuccal pocket but were scattered throughout the food pellet in the chamber.

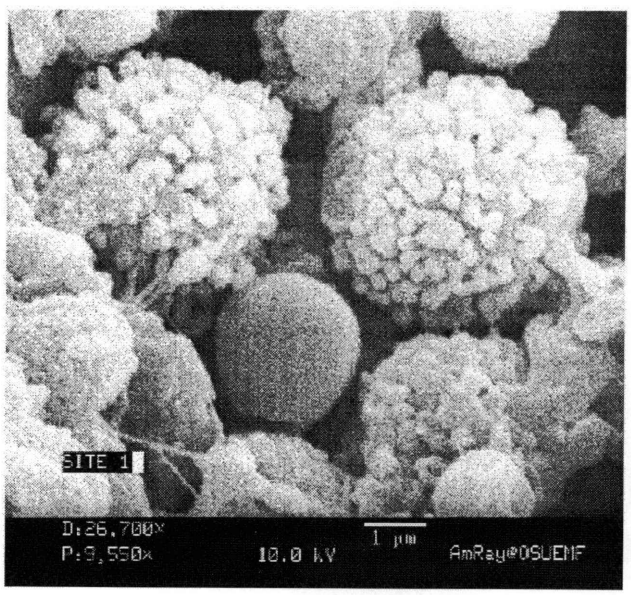

Figure 4.1: Interior of infrabuccal pocket at high magnification showing yeast-like cells.

4.4.2: Yeast isolation.

One hundred and fifty-five isolates representing 18 taxa were isolated from *Camponotus vicinus* workers, ant nesting material (substrate), frass under the colony and detritus and soil near the nests (Table 4.1). Most isolates were obtained from colony material or from the surrounding soil.

Seventeen isolates representing six different taxa were obtained from the infrabuccal pocket contents. The most commonly isolated yeast, *Debaryomyces polymorphus*, was found in all areas sampled, but occurred predominantly in the substrate and infrabuccal pocket. This species was found throughout the year in each colony surveyed (Figures 4.2A and 4.2B), but it was more frequent in July and October than in January. This yeast was also found consistently in substrate material and frass under the colonies during these two months and appeared to be more common in colonies collected from McDonald-Dunn Research Forest than in those from Philomath (Figure 4.2B).

Pichia guillermondii was found in all samples as well, but was less common in the infrabuccal pocket. Four *Cryptococcus* species—*C. luteolus*, *C. terreus*, *C. laurentii,* and *C. tsukubaensis*—were frequently isolated from soil. *Cryptococcus tsukubaensis* was isolated twice, both times from the substrate.

We isolated several species of *Bulleromyces* from the soil. *Fellomyces fuzhouensis*, *Rhodotorula glutinus*, and *Debaryomyces hansenii* were isolated from substrate, frass, and soil. *Candida edax* was isolated from all samples, but it occurred more often in the substrate and soil than on the ants. *Candida ergastensis* was found in the infrabuccal pocket, substrate, and frass. *Bulleromyces* was predominantly in the soil, with only one isolate in the infrabuccal pocket. Similarly, *Cryptococcus laurentii* was isolated only once from the infrabuccal pocket and once from the soil.

Table 4.1: Frequency of yeasts isolated from the carpenter ant *Camponotus vicinus* and surrounding habitats.

	Isolation frequency					
Isolate	Infrabuccal pocket	Exoskeleton	Substrate	Under	Soil	Isolate total
Debaryomyces polymorphus	10	5	12	8	2	37
Pichia guilliermondii	2	3	4	2	5	16
Cryptococcus luteolus	0	0	2	1	10	13
Bulleromyces spp.	1	0	0	3	9	13
Fellomyces fuzhouensis	0	0	4	1	8	13
Candida edax	1	1	3	2	3	10
Rhodotorula glutinus	0	0	0	1	8	9
Cryptococcus terreus	0	0	1	1	6	8
Debaryomyces hansenii / *Candida famata*	0	0	2	2	4	8
Candida ergastensis	2	0	2	3	0	7
Filobasidiella neoformans	0	0	0	2	5	7
Zygoascus hellenicus	0	0	2	1	0	3
Cryptococcus laurentii	1	0	0	0	1	2
Cryptococcus tsukubaensis	0	0	2	0	0	2
Debaryomyces maramus	0	0	1	0	1	2
Rhodotorula aurantiaca	0	0	0	0	2	2
Rhodotorula pustula	0	0	1	0	0	1
Sporidiobolus pararoseus A	0	0	0	0	1	1
Total Isolations	17	9	36	27	66	155

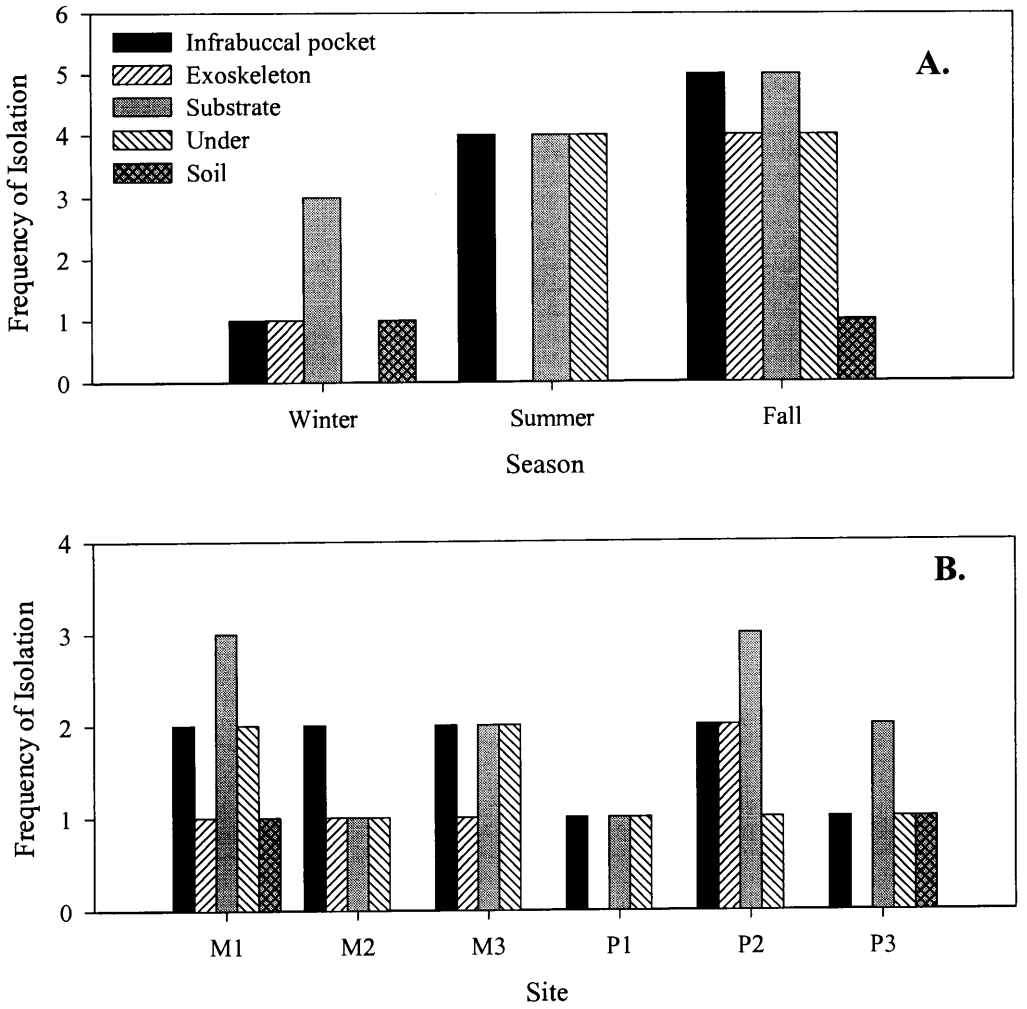

Figure 4.2: Effect of season on frequency of the yeast *Debaryomyces polymorphus* in six carpenter ant colonies (A). Occurrence of the yeast *Debaryomyces polymorphus* from six carpenter ant colonies over 1 year (B) (M = McDonald Forest; P = Philomath).

Filobasidiella neoformis, *Zygoascus hellenicus*, *Debaryomyces maramus*, *Rhodotorula aurantiaca*, *R. pustula*, and *Sporidiobolus pararoseus* A were found less frequently than other species, particularly *D. polymorphus* and *P. guilliermondii*, in substrate, frass and soil.

4.5: DISCUSSION

The limited isolations from the ant exoskeleton most likely reflect ant grooming behavior. Many ant species have functional metapleural glands that contain anti-microbial substances used to keep colonies free of microorganisms. However, the metapleural glands of *Camponotus* are atrophied, and it is not known how these ants keep themselves clean except by grooming (Wilson and Holldobler, 1990). Because ants were sometimes covered with colony material or frass, the few isolates found on the exoskeleton may be the result of contamination during sampling.

Several of the yeast species isolated from the infrabuccal pocket—*D. polymorphus, P. guillermondii*, *C. edax*, and *C. ergastensis*—have also been found associated with wood-boring insects and their frass (Barnett, et al. 1990). *Pichia guilliermondii* has also been found in floral pollen where it produces enzymes that aid in pollen digestion by honey bees (Gilliam, 1997). In addition, *D. polymorphus* has been isolated from the mounds of active *Formica rufa* colonies (Golubev and Bab'eva, 1972b). In our study, this yeast was frequently isolated from substrate, the infrabuccal pocket, and frass, but was rarely found in the soil outside of the colonies, suggesting that its development was related to favorable colony conditions.

Ba et al. (2000) found *D. hansenii*, *P. guilliermondii, Candida famata*, and *Cryptococcocus terreus* in the brood chamber soil in mounds of the imported red fire ant *Solenopsis invicta*. *Candida* species have been isolated from adult hemolymph and larval guts of *S. invicta* (Ba and Phillips, 1996). We found *D. hansenii* on substrate, but never isolated it from the ants themselves. Even though *D. hansenii* is reported from a wide variety of habitats, it is not known to occur on or in wood (Barnett, et al. 1990).

Many *Cryptococcus* species commonly occur in the soil, and it was interesting to find them associated with the infrabuccal pocket and ant galleries

(Barnett, et al. 1990). Soil and detritus contain increased amounts of nutrients that are capable of supporting more species of yeast.

Although *Candida edax* was isolated predominantly from soil, this species is commonly associated with insect frass and wood, as are many species of *Rhodotorula* (Barnett, et al. 1990). *Rhodotorula pustula* is commonly found on fruit, and the significance of its presence in the ant colonies is unknown. *Debaryomyces polymorphus* was isolated from substrate material, frass under the colonies, and, once, from outside the colonies. It was the most common infrabuccal pocket yeast in this study, found throughout the year in each colony surveyed. Interestingly, *D. polymorphus* was found in the infrabuccal pocket in the January; because ants do not feed in winter, this finding suggests that the fungus had been retained in the infrabuccal pocket. Generally, ants do not retain infrabuccal material for long time periods (Eisner and Happ, 1962). *Debaryomyces polymorphus* was isolated once in January and once in October from soil in different locations, but it was found in all colonies sampled.

Debaryomyces species are also found in the intestines of xylophageous beetles, where they comprise 50%–85% of the microflora (Gusteleva, 1975). These yeasts are active producers of the B vitamins—biotin, thiamin, pyridoxine—and nicotinic and pantothenic acids, which they can synthesize more intensively than can bacteria associated with xylophageous insects (Gusteleva, 1975). Ba and Phillips (1996) found that fire ant colonies with yeasts weighed more than those without, suggesting that these organisms aided in colony nutrition. Ba et al. (1995) also found that the yeasts *Candida parapsilosis* and *Yarrowia lipolytica* synthesized ergosterol and zymosteroil in the larvae of *Solenopsis*. Yeasts can aid in the breakdown of toxins, aiding in the diets of certain fruit flies (Starmer, et al. 1986), and can produce chemicals that modify insect behavior (Leufven and Nehls, 1986). The close relationship between yeasts, wood-inhabiting insects, and other ant species, as well as the common presence of *D. polymorphus* in colonies of *C.*

vicinus, implies that carpenter ants may utilize yeast as a source of nutrients or enzymes that aid in the metabolism of dietary components in the infrabuccal pocket.

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CHAPTER 5: EXAMINATION OF AN ARTIFICIAL DIET, EFFECTS OF NUTRIENT DELETION AND EXPOSURE TO LIVE YEAST ON LARVAL DEVELOPMENT OF DEFAUNATED AND NON-DEFAUNATED *CAMPONOTUS VICINUS (MAYR).*

Mark Mankowski and J.J. Morrell

5.1: ABSTRACT

The effects of an artificial diet, defaunation, and exposure to live yeast on larval development were examined in the carpenter ant *Camponotus vicinus* (Mayr). Diet significantly affected the growth of larvae and number of adult ants produced in a vitamin deletion test, but ants sometimes developed better on incomplete diets. Heat exposure significantly affected larval development, while exposure to the yeast *Debaryomyces polymorphus*, also significantly affected larval development and the number of pupae and adults produced. Yeasts may have helped ants to more efficiently assimilate nutrients when fed nutrient-deficient diets.

5.2: INTRODUCTION

Carpenter ant workers possess a buccal filter or infrabuccal pocket that limits the ants' ability to pass large food particles to the midgut (Forbes, 1938; Eisner and Happ, 1962). Food filtered in the buccal cavity forms a pellet that is eventually evacuated. Febvay and Kermarrac (1986) suggested that some extracelluler digestion begins in the buccal chamber via enzyme action. The pellet is squeezed by muscular action and any liquid produced passes to the midgut. An abundance of microorganisms identified as gram-negative bacteria and several

unidentified yeast species have been found in the infrabuccal pocket of these ants, but the role of these microorganisms is unknown (Hansen, et al., 1999, Chapter 4). Microorganisms serve as food sources for many arthropods (Martin and Kukor, 1984). Ingested microorganisms can be digested and contribute to the nutritional requirements of the organism that ingests them. Aside from their nutritive value, surviving microorganisms can remain active in the gut tract and contribute to the digestive or metabolic system of the organism that consumes them (Martin and Kukor, 1984). Kaufman et al. (2000) noted that many of the relationships between insects and gut microorganisms are facultative and involve entire communities of organisms that can differ with food source.

Microorganisms in the gut tract of insects may aid in the synthesis of B vitamins and sterols that are necessary for proper metabolic functioning and larval development (House, 1954; Dadd, 1961; Baker, 1975; Armstrong, 1978; Wakayama et al., 1984). Most studies used artificial (holidic) diets complete with all components in known amounts, then delete various components to examine the effects on insect growth compared to the complete or basal diet. Most of these experiments assessed the larvae of non-social insects and measured larval growth and reproductives produced. Experiments examining the role of nutrition on the development of individual ant larvae are difficult since larvae are not kept in individual cells but rather groups where one cannot be distinguished from the other (Wheeler, 1994). Few studies examine the effects of an artificial diet and component deletion on ants. Smith (1942; 1944) used a meridic diet, a diet where some quantities of the components are unknown, supplemented with yeast extract to supply vitamins to carpenter ants and found that the absence of yeast extract affected the stature of brood and workers in the colony. Normal colony growth in *C. pennsylvanicus* required at least the vitamins supplied by brewers yeast. Ant survival on the vitamin free diet was attributed to the activity of microorganisms in the ants, however, the nutritional association of *Camponotus* with yeasts or other microorganisms has not been examined.

Yeasts of the genus *Debaryomyces* are associated with the nests of the forest ant *Formica rufa* and are known to generate B vitamins in the guts of some wood boring beetles (Golubev and Bab'eva, 1972a and 1972b, Gusteleva, 1975). Associations between *Formica* and this yeast were not characterized, but it was suggested that an association existed since the yeasts were found only in active ant colonies. This yeast is also commonly associated with the carpenter ant *Camponotus vicinus* (Mankowski and Morrell, in revision). Ba (1995, 1996, 2000) found that yeasts associated with colonies and larval guts of the fire ant *Solenopsis invicta* appeared to contribute to overall colony health and produced sterols that the ants may use in nutrition since insects cannot generate sterol compounds (Dadd, 1977, Rienecke, 1985). Yeast-like endosymbionts are present in the digestive systems of many beetles (Pant and Dang, 1972, Gusteleva, 1975) and pass into the beetle larvae as early as the egg stage. Yeasts can also serve as more facultative ectosymbionts. Adult Chrysopid lacewings that exist on honey-dew obtain nutrients from *Torulopsis* yeasts in their digestive tract (Hagen and Tassen, 1972). Many other insects have associated microorganisms that synthesize required nutrients absent from their diets such as B complex vitamins or sterols (Dadd, 1977). Yeast species can synthesize B vitamins, particularly thiamine, nicotinic acid and biotin, and sterols on minimal media.

Test organisms are often rendered aposymbiotic or defaunated (without microorganisms) in studies using artificial diets to examine the effects of vitamin deletion. Disturbance of microbial gut flora by chemotherapy or sterile rearing have supported the hypothesis that these microorganisms aid in host nutrition (Santo Domingo et al., 1998). No studies, however, have examined the effects of a completely holidic diet on carpenter ant brood development, the effects of defaunation on microorganisms in the buccal cavity or exposure of larvae to a live yeast commonly found in the infrabuccal pocket and in wild carpenter ant colonies.

The primary objectives of this research were to develop an artificial diet for the carpenter ant *Camponotus vicinus* and examine the effects of component

deletion from this diet on larval growth. This diet or a variation of it was used to test the hypothesis that *Debaryomyces polymorphus*, contributed to the development of brood in deafaunated and non-defaunated *Camponotus vicinus*.

5.3: MATERIALS AND METHODS

5.3.1: Effects of a holidic diet and nutrient deletion on *C. vicinus*.

5.3.1.1: Artificial Diets:

Artificial diets were prepared as follows: Stock solutions of the vitamins and minerals were added to the various diets in 1 ml aliquiots. The ribonucleic acid (RNA) solution was prepared by adding 2 ml of 1M KOH to 3 ml of hot water and then pouring this solution over the ribonucleic acid dissolve it. A 1 ml rinse with warm sterile water was used to remove any remaining solution.

Fatty acids and fat-soluble vitamins were weighed in 5 ml beakers and dissolved with 2 ml of hot ethanol. This mixture was poured into 80 ml of hot water to which 0.5 ml of Tween 80 had been added. The solution was adjusted to 100 ml with hot sterile water. Forty milliliters of this solution was used per 100 ml of diet and the amounts of fatty acids were adjusted accordingly (Table 5.1).

The diets tested were based upon previous studies (Yazgan, 1972; Dadd, 1961; House, 1954). Each diet was prepared by adding dry amino acids to a 250 ml flask. The amino acids were dissolved with 40 ml of the fatty acid solution along with 9 ml (one of each) mineral salt and 6 ml of the RNA solution. We then added 1.5 ml of 1M KOH to adjust the pH to 6.5. The mixture was stirred for 10 minutes and autoclaved at 120° C for 15 minutes then allowed to cool.

The water soluble portion of the diet was prepared by placing 10 grams of sucrose in a 50 ml beaker and adding 1ml of each vitamin from the stock solutions. This dissolved the sugar and the mixture was placed in a syringe and water added to 42.0 ml. We added 1.5 ml of 2N K_2PO_4 to adjust the pH to 6.5. This solution was then filtered through a 0.22μ filter into the non-water soluble portion.

Table 5.1: Composition of the complete basal diet tested on carpenter ants.

Component	mg/100ml diet			Component	mg/100ml diet
Amino acids				**Fat Soluble**	
Alanine	100			Cholesterol	50
Arginine	100			Linoleic acid	25
Aspartic acid	100			Linolenic acid	20
Cysteine	30			Oliec acid	60
Glutamic acid	200			Palmitic acid	60
Glutamine	50			Tween 80	500
Glycine	150			Vitamin A	0.1
Histidine	50			Vitamin E	1.0
Hydroxyproline	30				
Isoluecine	100			**Water soluble**	ug/ml diet
Luecine	150			Ascorbic acid	100
Lycine	100			Amino benzoic acid	25
Methionine	50			Biotin	2
Phenylalanine	100			Calcium Pantothenate	50
Proline	150			Choline Chloride	1,250
Serine	100			Folic acid	25
Threonine	75			Inositol	250
Tryptophane	75			Nicotinic acid	100
Tyrosine	100			Pyridoxine	25
Valine	100			Riboflavin	25 (2.5 2001)
Inorganic Salts				Thiamine	25
$CaCl_2$	10	$NaHPO_4$	5	Vitamin B12	1
$CoCl_2$ $6H_2O$	2	ZnCl	2		
$CuSo_4$ $5H_2O$	2			**Other**	mg/100ml diet
$FeCl_3$ $6H_2O$	8			Sucrose	10,000
K_2PO_4	50			Ribonucleic acid	100
$MgSO_4$ $7H_2O$	60			KOH	1.5
$MnSO_4$ H_2O	0.5			K_2 PO_4	1.5

5.3.1.2: Nutrient deletion

The effects of vitamin B deficiencies on brood development were tested using a methodology similar to that used by Armstrong (1978), Dadd (1961) and Smith (1944). Eleven groups from three colonies of *Camponotus vicinus* consisting of 12 workers and twenty 3rd to 4th instar larvae were removed from wild colonies in the winter and kept in cold storage until needed. Experimental colonies were mimics of small satellite colonies in that they were queenless. The ants and brood were placed in sterile petri dishes with a wet sponge for water and kept at 25° C. These fragmentary colonies were fed a basal diet that consisted of all the components listed in Table 5.1 (Smith, 1944; Armstrong, 1978, Yazgan, 1972). Ants were fed at 3-day intervals by placing 100 µl of the diet on a small tin foil square shaped into a cup. All feedings were performed in a laminar flow hood to minimize colony contamination.

The role of B vitamins in ant nutrition was tested by varying the basal diet to be deficient in one of the B vitamins (Table 5.1). Ascorbic acid was the only water-soluble vitamin that was not deleted at any point in the study. Vitamins were added in micrograms per gram of diet according to Dadd (1961). Apart from single vitamin deletion treatments, we also tested treatments where all B vitamins were excluded, cholesterol was removed, both B vitamins and cholesterol were deleted, a diet of sugar water (0.5M sucrose) only, and a diet of sugar water and all B vitamins.

The ants were incubated at 22-23° C for 12 weeks and monitored at three-day intervals when food was changed. Any pupae formed were removed prior to eclosion and their head widths measured using an ocular micrometer. Weight measurements of developing brood were made every two weeks and the number of brood and brood weights were recorded to assess the role of B vitamins on ant development.

5.3.2: Effects of diets supplemented with *Debaryomyces polymorphus* 2001.

5.3.2.1: Defaunation with heat and chemicals and exposure to yeast

The effects of adding live *Debaryomyces polymorphus* to some of the same diet treatments was assessed determine if removing yeasts from the infrabuccal cavity affected the stature of the ants when fed a normal basal diet and diets deficient in various B vitamins, and diets supplemented with live yeast. We first experimented with defaunating entire groups of ants by either feeding groups of worker ants 100 µl of a mixture of propiconazole and tetracycline or by heat-treating ants at 39° C for 48 hours. Tertracycline was used to eliminate endosymbionts in the ant midguts (Buchner, 1965). Propiconazole was used to inhibit yeast growth. Yeasts and fungi grew after feeding ants various concentrations of the propiconazole and tetracycline mixture and plating the contents of exposed ants infrabuccal cavities. Pant and Dang (1972) found that supra optimal temperatures stopped the growth of the yeast symbionts in the beetle *Sitophilus oryzae*. We tested elevated temperatures on *Debaryomyces polymorphus* and found that this species did not grow at 39° C. Fifteen ants were exposed at this temperature for 48 hours, then the ants were fed a dosage of 6.66E-4mg/ml of the propiconazole-tetracycline mixture in 0.5 M sucrose. Treated ants were dissected after one-week and the infrabuccal pocket contents were removed and placed in 190 µl of sterile PBS solution. Fifty microliters of this solution was plated on acid media and incubated for five days at 25° C. Plates were then examined for yeast growth. The absence of growth led us to conclude that the infrabuccal cavity was aposymbiotic.

Sixteen groups from two colonies of *Camponotus vicinus* consisting of 8 workers and fifteen second to third instar larvae were removed from wild colonies and treated as in Experiment 1. Four diets were tested: A basal diet, no B

vitamins, no B vitamins and no cholesterol, and sugar water only. The basal diet contained lower amounts of riboflavin (2.5 μg/ml) than in initial studies.

Half of the ants were exposed to yeast cultures every week. A suspension of *Debaryomyces polymorphus* was grown in 125 ml of vitamin free media on a rotary shaker at room temperature for five days and then filtered through a 0.22μ filter paper. The vitamin free media was made according to Barnett (1990) and contained no B vitamins or growth factors. The yeasts were removed from the filter paper and placed into 20 ml of sterile water. This yeast suspension was then squirted onto the worker ants and larvae in 100 μl aliquioits so that the workers would ingest the yeasts as they groomed themselves and larvae. Another group of 8 replicates per diet was exposed to 39° C for 48 hours then fed a mixture of propiconazole-tetracycline to destroy any yeasts harbored in the infrabuccal cavity. Heat and chemically treated ants were fed the same diets and exposed to the yeast as the non-heat or chemically treated ants.

For all treatments, ants and brood were placed in clean petri dishes with a wet sponge and tinfoil square formed into a small cup to hold the diet. In the initial artificial diet study, we measured brood weights, adult numbers and adult weights along with head capsule width and length of eclosing adults to assess treatment affects on the ants. Pupae were removed and kept at 25° C until the cuticle of the ant inside darkened. When this occurred the ant was physically removed from the pupal case and its weight, head width and length recorded. In the subsequent study, we measured the above variables along with pupal weights and numbers due to loss of many adult ants to mold and desiccation.

5.3.2.2: Heat and chemical test

During the test period, we noticed that larvae in the heat/chemical treatment had lower weights than non-treated replicates. We then performed a side study to

examine the effects of heat and chemicals on development. Four treatments of five replicates of 8 workers ants and 15 larvae were exposed at 39° C for 48 hours, exposed to chemicals only (fed 100 µl of 6.66E-4mg/ml of the propiconazole-tetracycline mixture in 0.5M sucrose three times per week), exposed to both heat and chemicals, and left untreated. Ants were fed at three-day intervals and larval development and adults were monitored every two weeks as described earlier.

5.3.2.3: Yeast isolations

At the end of the twelve-week period, selected ants were removed and dissected. The infrabuccal cavity contents were removed and placed in 190 µl of sterile PBS solution. Fifty microliters of this mixture was plated on acid medium and incubated at 25° C for five days. The plates were examined for any signs of yeast growth.

5.3.3: Statistical analysis

The initial tests were analyzed using ANOVA procedures in SAS (SAS, 2000). The subsequent experiment was analyzed as a three way multifactorial ANOVA between diet, heat/chemical treatment and yeast exposure. We analyzed all variables with a general linear models procedure, using least square means to compare any significant means.

5.4: RESULTS

5.4.1: Nutrient deletion 2000

5.4.1.1: Brood weights:

Brood weights for each treatment over the experimental period are shown in Figure 5.1. Some diets appeared to greatly inhibit brood growth, but the majority of the single B vitamin diets did not seem to affect brood development and most did better than the basal diet. Deletion of calcium pantothenate and pyridoxine were the only single B vitamin deletions associated with lower brood weights than the basal diet. Deletion of cholesterol or all B vitamins did not appear to greatly affect brood weights, however, the deleting both cholesterol and B vitamins from the same diet appeared to hinder brood development. Brood also developed poorly on sugar water only and sugar water with all B vitamins.

Final brood weights at 12 weeks were a significantly affected ($p<.0001$) by diet (Figure 5.2). Although the basal diet did not perform as well as the majority of the single vitamin deletion tests, only the no thiamine treatment had brood that were significantly higher in weight ($p=.04$). There was some evidence ($p>.05<.10$) that ants fed inositol produced lighter brood. Brood weights for treatments without nicotinic acid, all B vitamins, calcium pantothenate, and sucrose only were lower than the basal diet, but the differences were not significant. Brood weights for colonies fed diets of sugar water with B vitamins, and a diet without both B vitamins and cholesterol were significantly lower than the basal diet ($P<.05$).

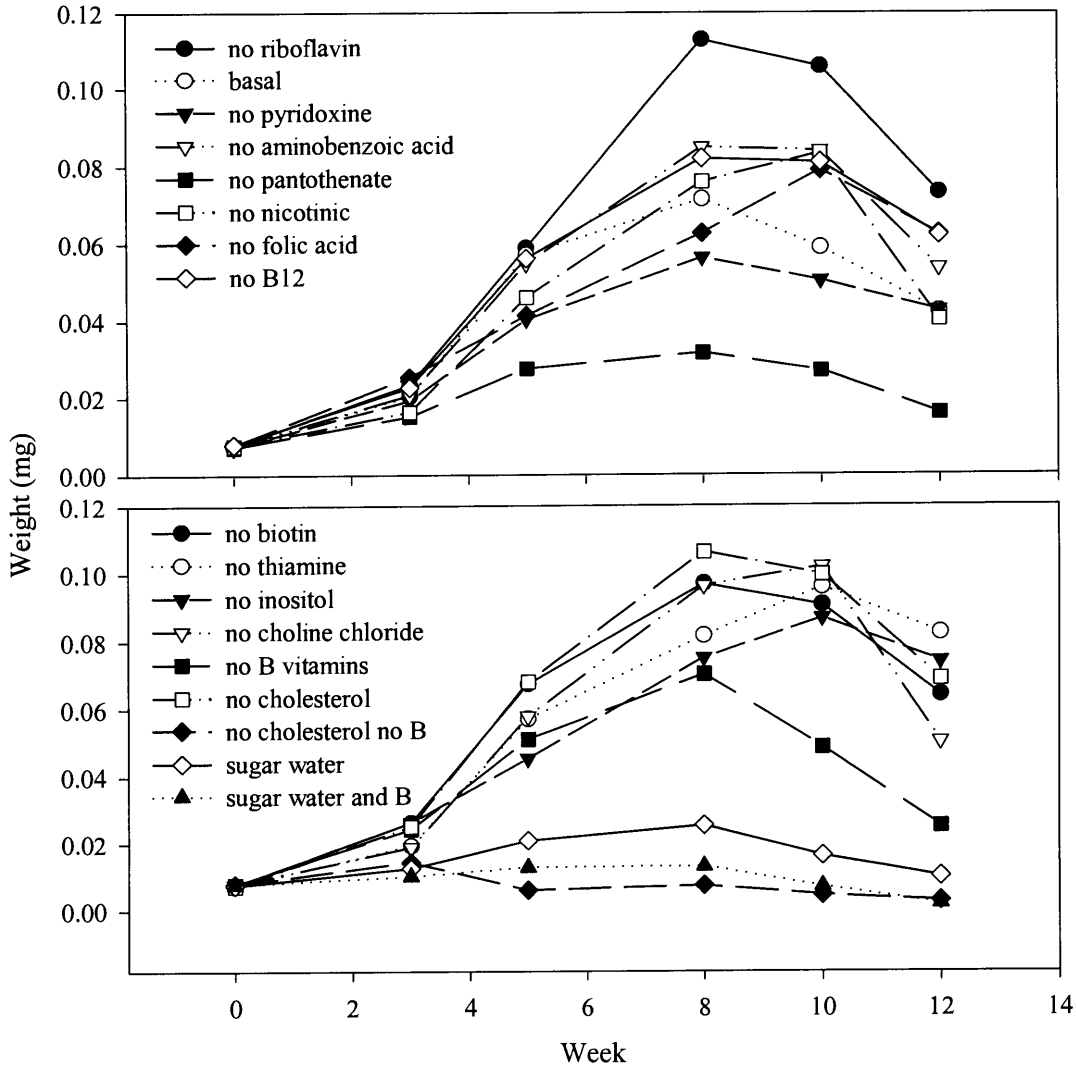

Figure 5.1: Brood development over 12 weeks for *Camponotus vicinus* fed several artificial diets with various components deleted.

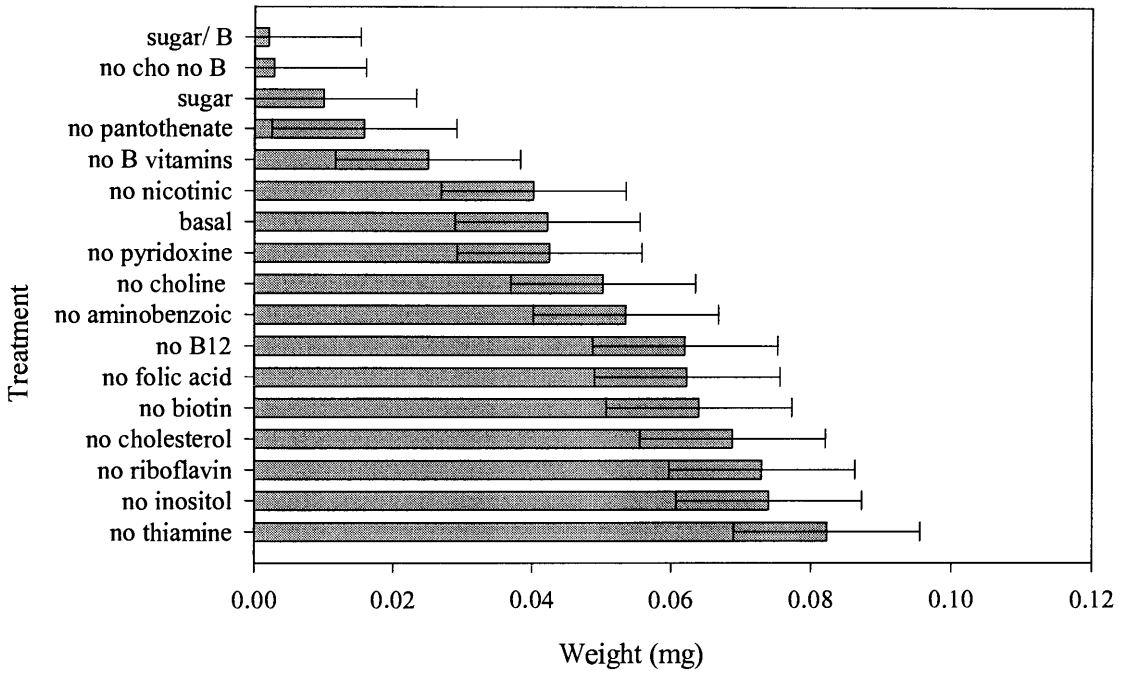

Figure 5.2: Total brood weight at the end of 12 weeks for colonies of *Camponotus vicinus* fed several different artificial diets with various components deleted.

5.4.1.2: Adult numbers:

Diet significantly affected ($p<.0001$) the number of worker ants produced (Figure 5.3). There was strong evidence ($p<.05$) that colonies fed diets without riboflavin, thiamine, choline chloride, and cholesterol produced significantly more adult ants than the basal diet. There was some evidence ($P>.05<.10$) that ants feeding on inositol, biotin aminobenzoic acid, and folic acid produced more workers than the basal diet. Diets lacking vitamin B12, pyridoxine, nicotinic acid and all B vitamins produced more adults than the basal treatment, but the differences were not significant. The basal diet minus calcium pantothenate was

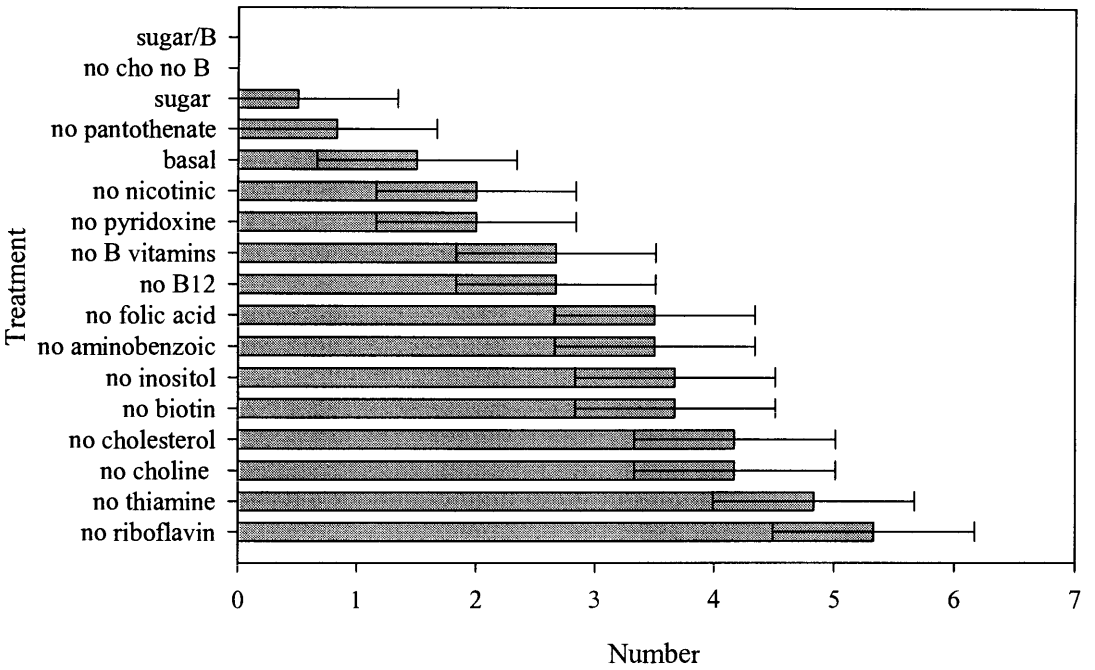

Figure 5.3: Number of adult worker ants produced after being fed various diets for 12 weeks.

the only single B vitamin deletion diet where colonies produced fewer adults than the basal diet. Ants fed sugar water also produced fewer workers than the basal diet. Diets of sugar water with all B vitamins and the diet without both B vitamins and cholesterol produced significantly fewer workers than the basal diet and diets lacking all B vitamins or cholesterol. No significant differences in total weight, head width, or length of the adult ants produced were found between treatments. Average worker weight was 0.01 mg, head width was 1.31 mm and length was 7.36 mm for the 269 worker ants produced for all treatments.

5.4.2: Effects of heat and chemical treatments and diets on ants exposed to *Debaryomyces polymorphus*

5.4.2.1: Brood weights:

There was a significant interaction between diet and heat/chemical treatment for total brood weight. (P= 0.006) Although the interaction between yeast exposure and diet was not statistically significant (p= 0.12), there was an interesting trend with this interaction that led us to retain it in our model. Figure 5.4A shows brood development over time for ants exposed to heat and chemicals. Brood in the heat/chemical treatments had lower weights throughout the experiment for the four diets (Figure 5.4B). Colonies fed the basal diet alone produced significantly heavier brood than all other treatments. All treatments exposed to heat and chemicals produced lower weight brood than those not exposed. The basal, no B vitamin, and no B vitamin/no cholesterol treatments exposed to heat and chemicals produced brood weights that were significantly lower ($p<.05$) than non-exposed treatments.

Figure 5.5A shows brood development for the interaction of ants fed yeast and the diets. There was some variation in development of brood exposed to the yeast (Figure 5.5B). Brood weight decreased for the ants fed the basal diet and a diet without B vitamins when ants were exposed to live *Debaryomyces polymorphus*. However, brood weight increasd for ants fed a diet without both B vitamins and cholesterol when these ants were exposed to live yeast.

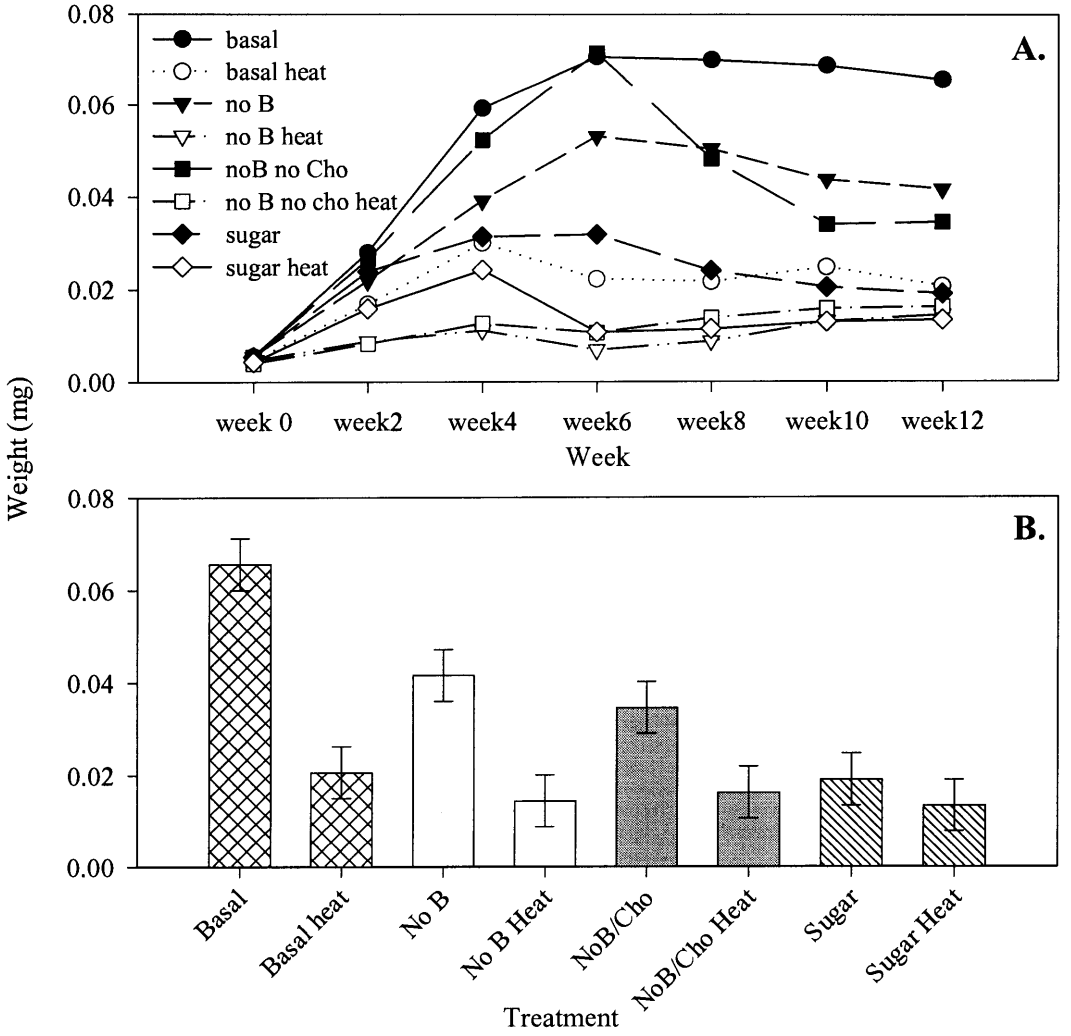

Figure 5.4: Brood weights over a twelve week period (A). Total brood weights (B) at the end of twelve weeks B for *Camponotus vicinus* fed four diets and exposed to 39°C for 48 hours then fed propiconazole and tetracycline.

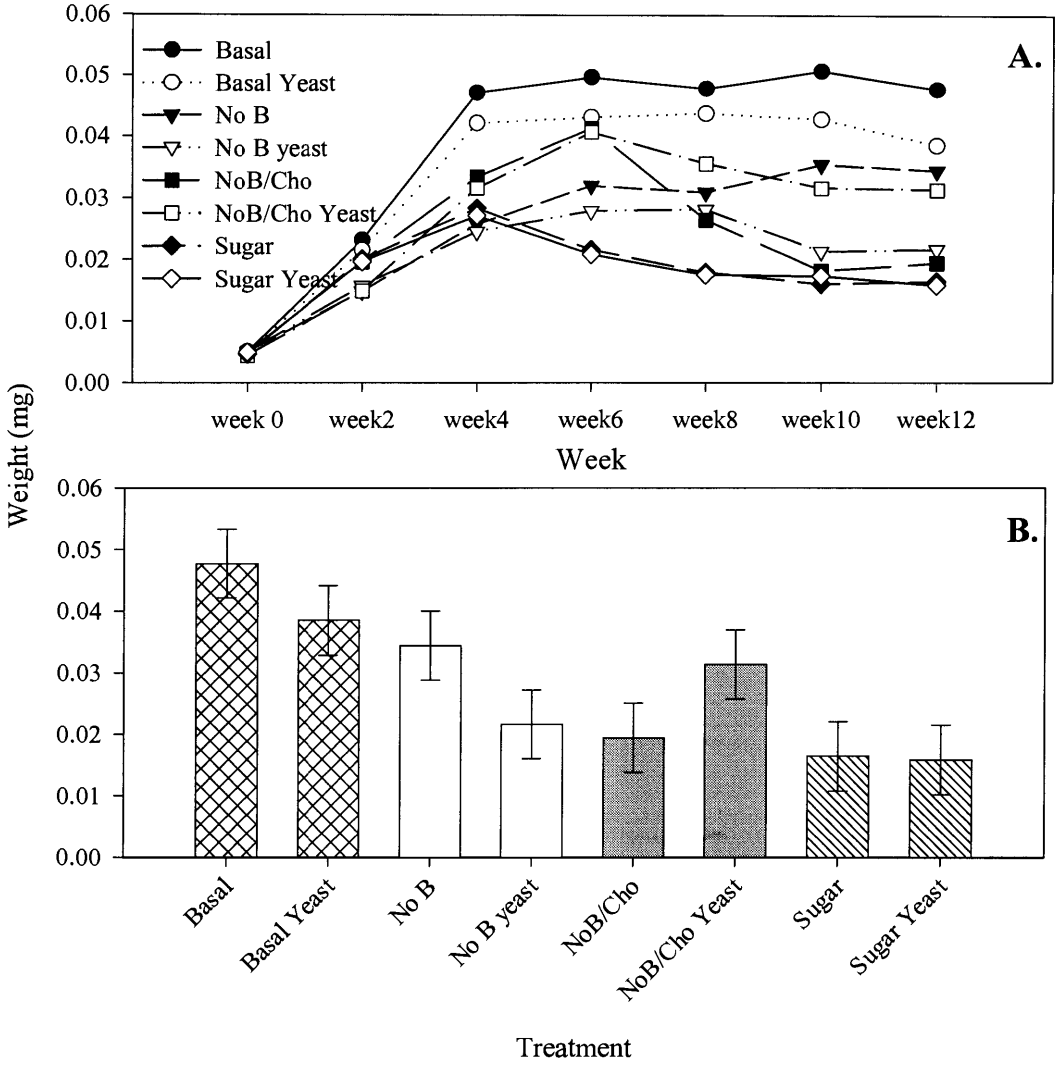

Figure 5.5: Brood weights over twelve weeks (A) and total brood weights (B) for *C. vicinus* fed one of four diets and exposed to live cultures of the yeast *Debaryomyces polymorphus*.

5.4.2.2: Pupal numbers and weight:

Figures 5.6A and 5.6B show the total number of pupae produced and the total weights of those pupae at the end of the experiment for colonies exposed to the heat/chemical treatment and fed one of the four diets. There was a significant interaction between heat/chemical treatment and diet for number of pupae ($p<0.02$) and pupal weight ($p<.0035$). Significantly more pupae were produced by ants fed the basal diet without heat or chemicals than all other treatments except the no B

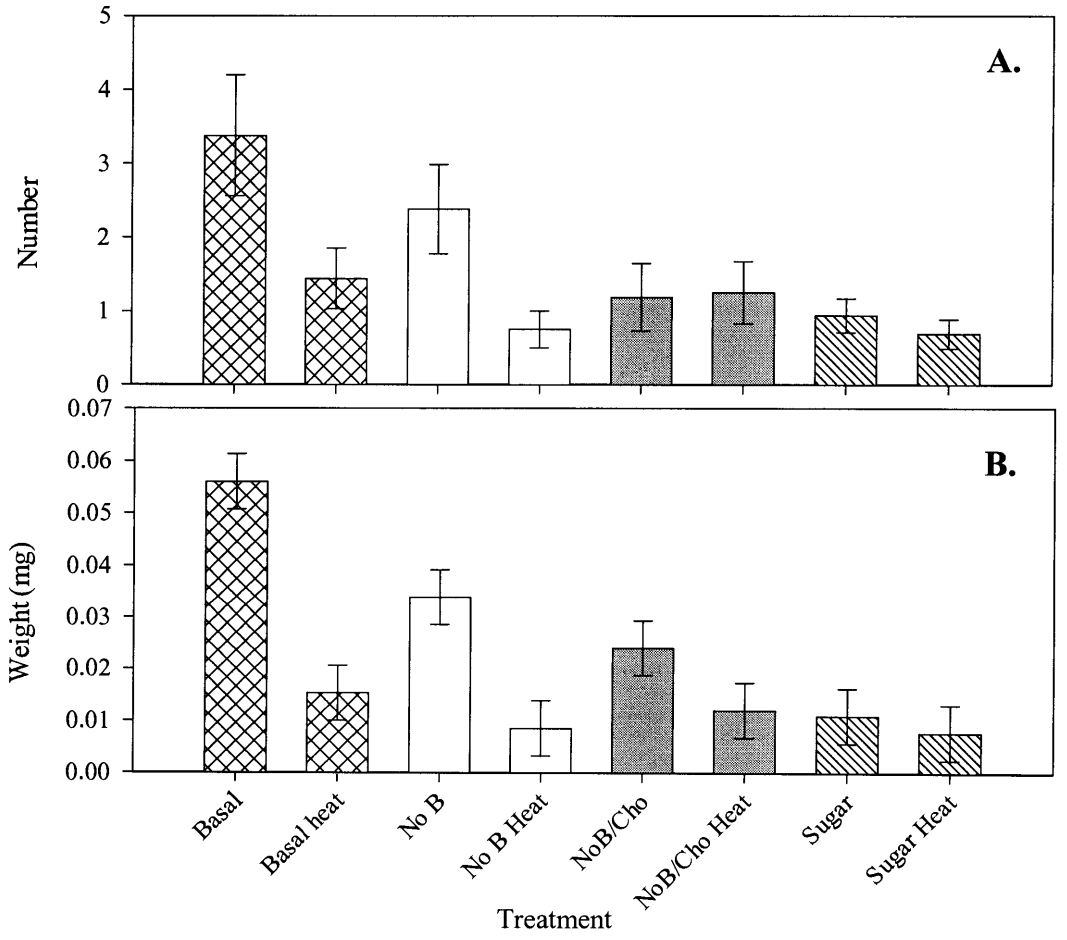

Figure 5.6: Effect of heat and diet on total pupal number (A) and total pupal weight (B) of *C. vicinus*.

vitamins without heat treatment. Ants exposed to the heat and chemical treatment produced significantly fewer pupae than the untreated ants fed the basal and no B vitamin diets. There were no significant differences in pupal number for ants fed a diet with no B vitamins, no cholesterol, and sugar water diets. A similar trend existed for pupal weights, except that ants fed the basal diet alone produced significantly heavier pupae than all other treatments.

Trends for pupal numbers and weights for colonies exposed to live yeast cultures were similar to brood weights (Figures 5.7A and 5.7B). The interaction of

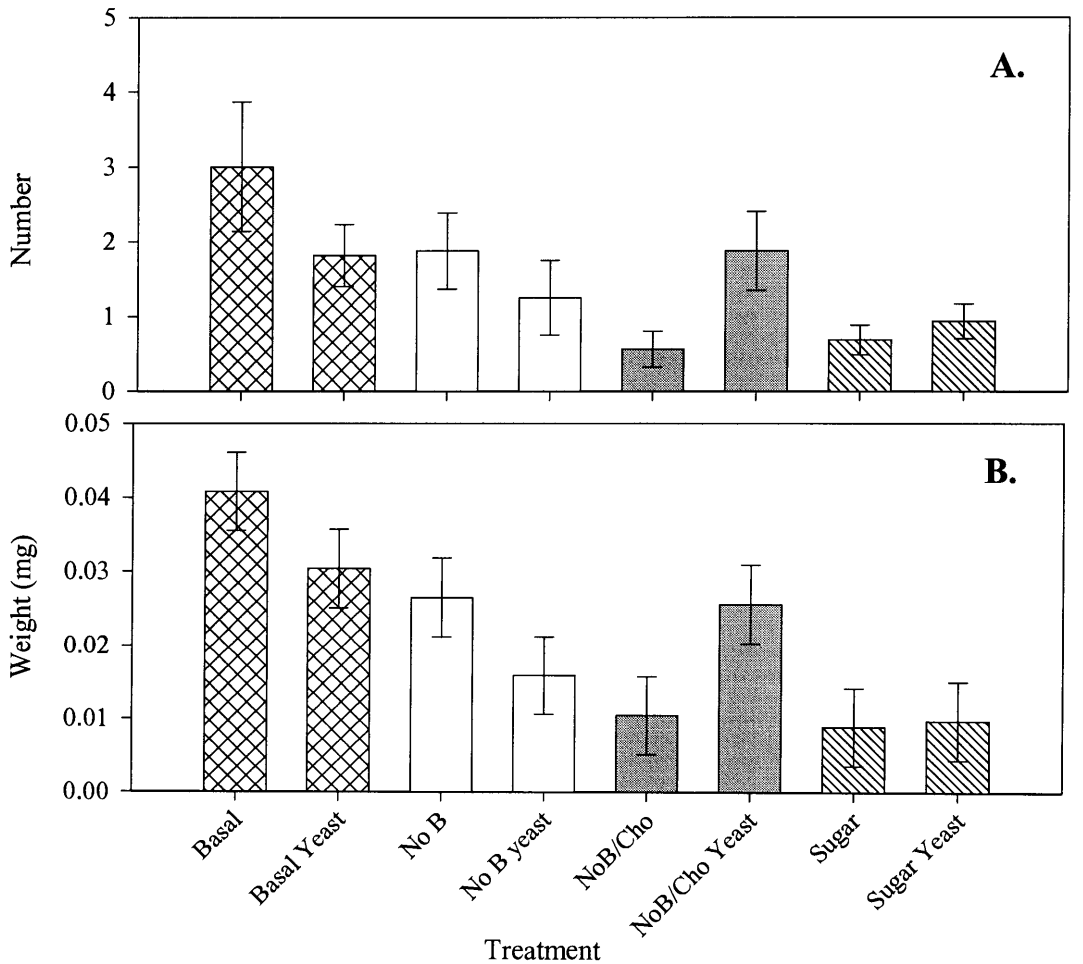

Figure 5.7: Total number of pupae (A) and total pupal weight (B) for *C. vicinus* exposed to *Debaryomyces polymorphus*.

yeast and diet was significant for number of pupae ($p<.0097$) and pupal weight ($p<.05$). The basal diet without yeast produced significantly more pupae than all other treatments. No differences were found between ants fed no B vitamins and sugar and those exposed to yeast. However, ants fed a diet lacking both B vitamins and cholesterol and exposed to yeast produced significantly more pupae ($p<.01$). Pupal weights were significantly higher for ants fed the basal diet without exposure to yeast compared to all the others except the basal diet with yeast. Ants fed no B vitamins and no cholesterol, but exposed to live yeast had significantly heavier pupae than those not exposed to yeast and fed the same diet.

5.4.2.3: Adult numbers and weight

No significant two- way interactions were found for adult number. However, we found a p-value of 0.11 for the interaction between yeast and diet indicating that although the interaction was not statistically significant, it may be suggestive of a trend biologically. Figure 5.8 shows the number of adult workers produced for ants fed diets with and without supplementation of live yeast.
We found a significant interaction of yeast supplementation and diet only for adult weights ($p< 0.03$). Workers fed the basal diet and exposed to yeast that were significantly heavier than those not exposed ($p= 0.005$) (Figure 5.9). There was also some evidence ($p= 0.06$) that ants fed a diet lacking cholesterol and B vitamins exposed to live yeast produced heavier workers than those not exposed to yeast.
We found significant ($p< 0.0001$) main effects of heat/chemicals and diet on adult head width and length. Workers exposed to the heat and chemical or non-heat chemical treatment had average head widths of 1.21 and 1.28 mm and lengths of 7.94 and 8.49 mm, respectively. The heat/chemical exposed ants had head widths and lengths that were significantly smaller ($p= 0.0001$) than unexposed

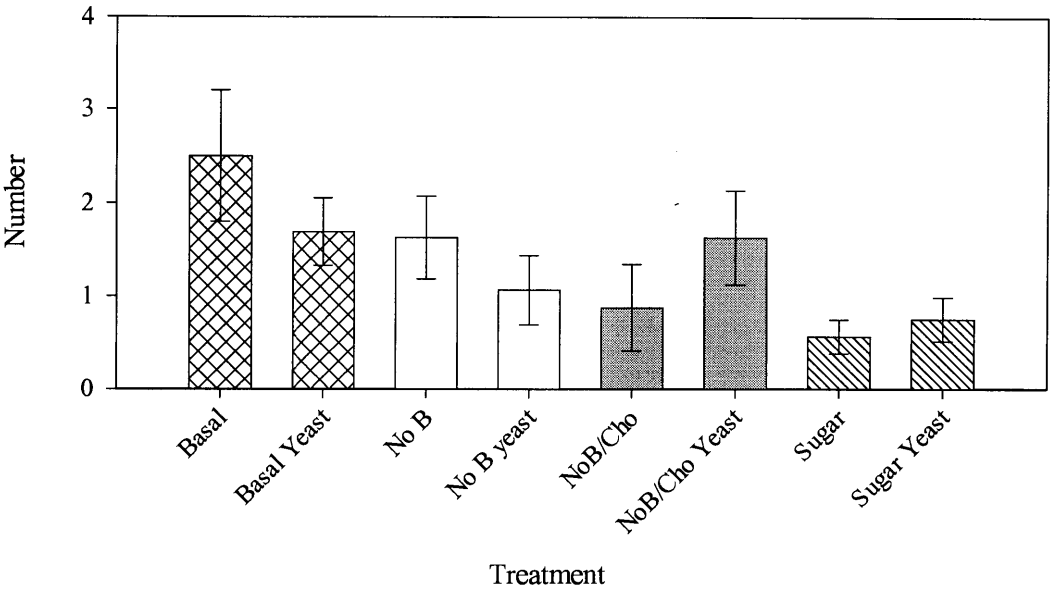

Figure 5.8: Effect of yeast and diet on number of adult workers of *C.vicinus* produced.

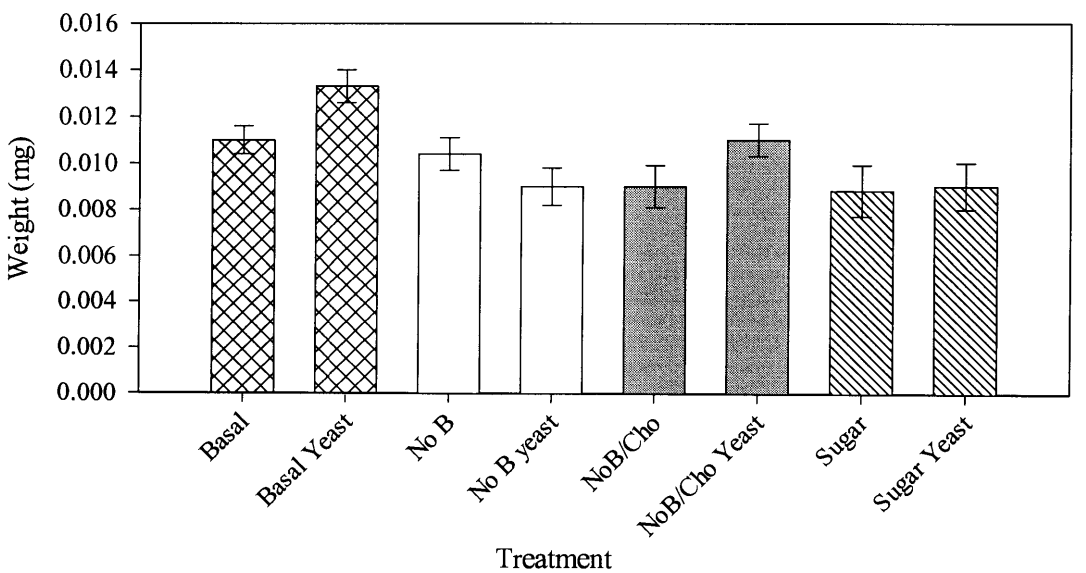

Figure 5.9: Effect of yeast and diet on the weights of adult workers of *C. vicinus* produced.

workers. Ants fed sugar water only had significantly smaller head widths than ants fed the other diets. Adult lengths were significantly shorter in treatments fed sugar water and no B vitamins compared with the basal diet.

5.4.3: Test of heat and chemical exposure:

Heat and chemical exposure significantly affected ($p < 0.001$) pupal number, pupal weight, brood weight, and adult weight. There was strong evidence that heat alone was the main cause of slow brood development in these ants. Ants exposed to heat alone produced significantly fewer pupae, adults and had lower total brood weight than treatments that received no heat or chemicals or chemicals alone (Figures 5.10a-d). When heat alone was compared to no treatment, we obtained p=values of 0.004 or less for the four variables graphed. Interestingly, we found no significant differences between treatments for adult weight, head width, and length.

5.4.4: Yeast isolations:

Plating of infrabuccal pocket contents at the end of the test period showed that workers in all treatments contained yeasts and other fungi. *D. polymorphus* was isolated from workers in all treatments except the basal diet treatments that were not exposed to yeast. Other yeast species and mold fungi were also isolated from these treatments including a yeast that appeared to be a *Candida* spp. (Barnett and Payne, 1990). This is not surprising since we did not try to keep the treatments or ants sterile throughout the experiment.

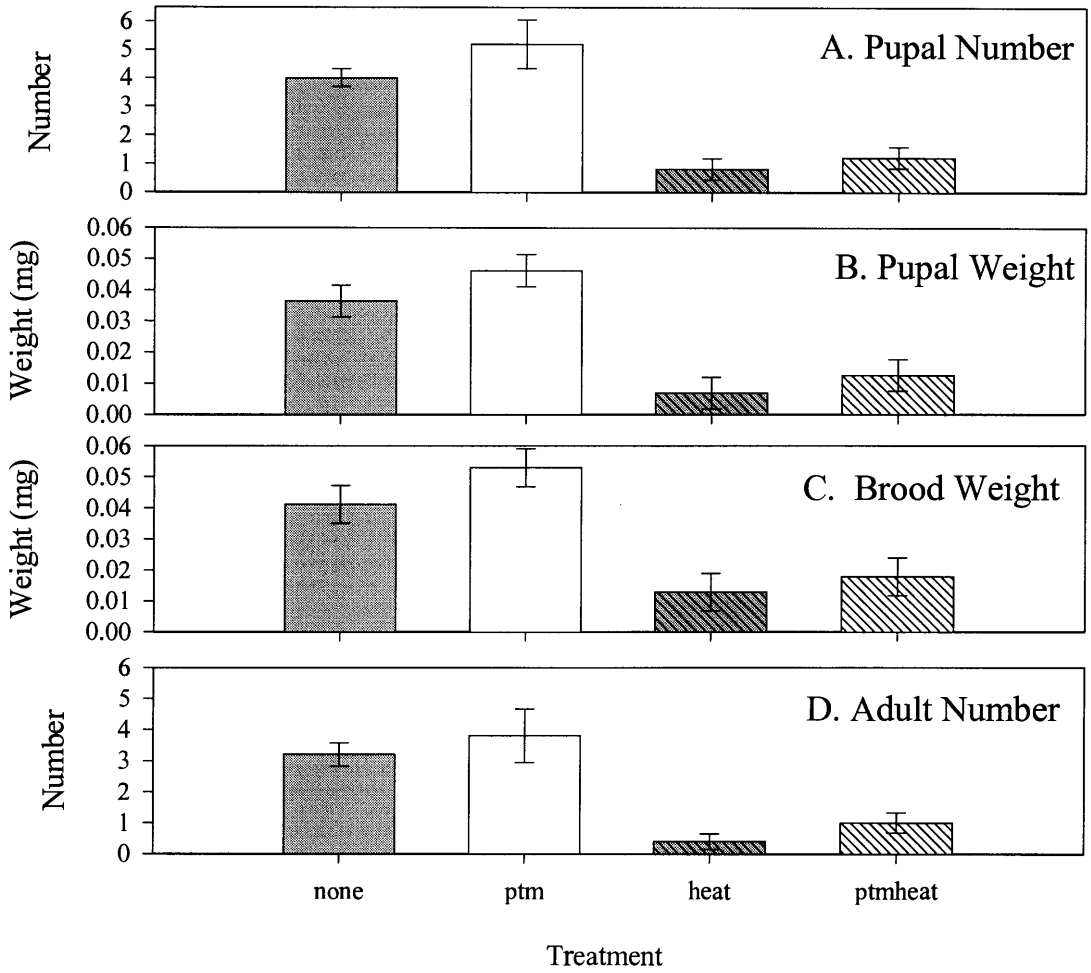

Figure 5.10: Effects of no treatment, heat, propiconazole-tetracycline (ptm), and both heat and propiconazole-tetracycline (ptmheat) on pupal number (A) pupal weight (B) total brood weight (C), and adult number (D).

5.5: DISCUSSION

Deletion of certain dietary components affected larval development and number of adult ants produced. While workers and larvae continued to develop on our diet, it is unclear whether this media represented an optimum diet. The primary purpose of any holidic diet is standardized optimal growth and development from the complete diet (Reinecke, 1985). Thus, a basal diet with all components infers

optimal nutritional capabilities and insures the best brood development. Results of our first years diet study indicate that the basal diet used was not optimal and in fact appeared to negatively affect brood development compared to diets that had growth factors removed. Developing an optimal artificial diet can be formidable. Limitations of holidic diets are that they must insure optimal growth, development and reproduction under sterile conditions that are often difficult to maintain. Each nutrient in the diet must also be present at a level that meets the requirement for structural materials and allows efficient metabolism of the other nutrients in the diet. A deficiency in one essential nutrient can affect the utilization rate of other nutrients and thus act as an anti-metabolite (Friend and Dadd, 1982, Reinecke, 1985). Conversely an excess of a nutrient can act as an anti-metabolite, hindering the absorption and assimilation of other nutrients. The diet may also be poorly digested because certain enzymes or factors are blocked or not present to allow digestion (Reinecke, 1985).

It is possible that the basal diet used in 2000 was too concentrated especially in the amounts of B vitamins since a diet containing only sugar water and B vitamins was even more detrimental to the ants than the basal diet. Since brood weights were higher and more adults were produced in the no riboflavin diet, we decided to greatly lower the amount of this vitamin in the second study. Low riboflavin level appeared to improve the performance of the basal diet. Uncertainties exist when examining the effects of B vitamin requirements because these compounds are difficult to completely eliminate from the test subject. Vitamins are also catalytic and can function in very small amounts (Rienecke, 1985). The situation is further complicated by the presence of microorganisms or symbionts capable of generating some of these substances. Dadd (1977) found that insects have general requirements for thiamine, riboflavin, pyridoxine, biotin, folic, and pantothenic acid. In some cases, vitamin requirements can only be shown if the test species is reared for multiple generations. This is extremely difficult in carpenter ants that undergo a diapause for almost six months each year.

In our study, only pantothenic acid deletion was associated with lower brood weights and lower adult numbers than the basal diet. Microorganisms in the ants could be generating vitamins or nutrients that, combined with the diet, and may have made it more indigestible. It is also important to note that most diet studies measure the number of reproductive forms produced as in indication of diet suitability. Although adult drones developed (10 out of 289) in colonies fed our diets, the lack of large workers and female reproductives indicates that our artificial diet was not assimilated very well by the ant larvae. Further experimentation with this diet would expand our limited knowledge of formicine nutrition.

Heat and chemical treatments appear to have deafuanated the infrabuccal cavity for a limited amount of time, since yeasts and other fungi were found in the buccal cavities since at the end of the experiment. It was difficult to keep the experiment completely aseptic and, thus, the ants probably picked up yeasts and other fungi from the environment. Several types of yeast were found in the infrabuccal cavites of both treated and non-treated ants. The presence of these yeasts might be an indication of stress associated with the experimental conditions (Gilliam, 1997).

Heating ant larvae at 39° C for 48 hours appeared to greatly inhibit larval development on all diets. The supra-optimal temperature may have stressed the larvae physiologically or it may have killed midgut endosymbiotic bacteria that aid larval nutrition. Pant and Dang (1972) used high temperature to defaunate beetle symbionts and found exposure to 33° C eliminated their yeast-like symbionts. Cassill and Tschinkel (2000) reported that prolonged temperatures did not affect fire ant worker size, but their studies used lower temperatures. They also found that workers exposed to higher temperatures fed larvae less but were more active so larvae still obtained the same amount of food as at lower temperatures. We do not know why larvae developed so poorly when exposed to heat in this study. Carpenter ant colonies are frequently found under detritus in areas exposed to full sun, but how they regulate optimal temperature for larval development is not known.

Exposure of some of the satellite colonies to live *Debaryomyces polymorphus* affected number of pupae and pupal weights of replicates that were fed diets lacking vitamins and cholesterol. Interestingly, brood in ants exposed to a diet lacking all B vitamins and exposed to live yeast were not affected, suggesting that the yeast may supply sterol compounds that aid in brood development. Unlike vertebrates, which biosynthesize sterols directly from acetate, insects have no way of synthesizing sterol compounds and must obtain them from their diet or microbial symbionts (Friend and Dadd, 1982). Sterols are also necessary for proper molting to occur in insects and, thus, are necessary for development and growth. Although the yeast may have helped the brood in the treatments with no cholesterol and B vitamins, ants fed sugar water only and sugar water and yeast did not differ. Sucrose treatments also lacked B vitamins and cholesterol, but there was little or no difference between the ants exposed and those not exposed to yeast. The effects of exposure to live yeast also was apparent in the adult weights of worker ants fed either the basal diet or the diet of no B vitamins and no cholesterol. Significantly heavier workers were produced when exposed to live yeast compared to colonies not exposed. The role of various nutrients like B vitamins and sterols in ant development has received little attention. Ba et al. (1995) measured sterol levels in fire ants and concluded that fire ants may obtain ergesterol from associated midgut yeasts. Maurer et al. (1992) examined sterols associated with leaf-cutting ants and found that the ants obtained sterols from their fungal symbiont. Smith (1944) found that carpenter ant colonies reared smaller brood on diets without yeast extract, indicating that yeast or something produced by yeast was beneficial to brood development.

Yeasts associated with other insects can produce enzymes that aid the insect in digestion of a food source and can produce or convert chemicals that alter insect behavior (Starmer et al., 1986, Leufven et al., 1984). The diet without B vitamins and cholesterol may have been less availiable to the ants as evidenced by the lack of development on this diet in the first years study. *Debaryomyces polymorphus* may produce enzymes that enhance digestibility of this diet to ant larvae. Starmer

et al. (1986) found several yeasts associated with desert fruit flies that aid in detoxification of compounds on the fruit fly host. Leufven et al. (1984) showed that yeasts convert aggregation pheromone to anti-aggregation chemicals in the trees attacked by the beetle *Ips typographus*.

The presence of *Debaryomyces polymorphus* may have affected larval development of *Camponotus vicinus* by producing nutrients or enzymes that facilitated larval digestion. Although we have only tested this for *D. polymorphus*, it must be noted that a more complex microbial community is probably involved in associations with ant nutrition and the infrabuccal pocket.

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CHAPTER 6: POTENTIAL FOR BIOLOGICAL CONTROL OF THE CARPENTER ANT *CAMPONOTUS VICINUS* (MAYR) USING THE ANT DECAPITATING FLY *APOCEPHALUS HORRIDUS* (BORGMEIER).

Mark Mankowski and J.J. Morrell

6.1: ABSTRACT

Feeding mixtures of propiconazole and tetracycline to *Camponotus vicinus* workers parasitized by the Phorid fly *Apocephalus horridus* indicated that high dosages of these chemicals resulted in more ant decapitation. Surveys of wild colonies indicated that few colonies were parasitized and the percent of workers parasitized ranged from 1-15%. Exposure of parasitized ant workers to 39° C for 48 hours resulted in no decapitation, indicating that the fly larvae were susceptible to this regime. Attempts to encourage lab-reared flies to attack groups of ants in the lab were unsuccessful.

6.2: INTRODUCTION

Carpenter ants are important degraders of wood used in structures and for utility poles in the Northwest (Shields, 1996). Carpenter ants are difficult to control because they do not use the wood as food source, instead excavating galleries in wood where they rear their young. While a variety of chemical treatments are applied to the ant galleries, these treatments do not always kill the queen (Akre and Hansen, 1995). This means that the colony can continue in some other part of a structure or pole. Previous efforts have evaluated the potential for using juvenile hormones to disrupt the colony, but getting the chemical to the

colony has proven difficult (Fowler and Roberts, 1982). Applying chemical barriers around each pole might limit carpenter ant attack, but the chemicals registered for this purpose are not long-lasting and would be expensive to apply on a regular basis.

One approach to arresting carpenter ant attack is to use biological agents. The classic approach to insect control is to identify fungi that can parasitize the insects. The most common fungi for this purpose are members of the genus *Bauveria*. These fungi invade the adults or larvae, where they grow through and eventually kill the host. Complete control of carpenter ants using this fungus would be difficult owing to the inability to deliver the fungal spores directly into colonies. One alternative to fungal control is to identify other insects that parasitize carpenter ants.

Ant decapitating flies in the genus *Apocephallus* lay their eggs on the backs of carpenter ant workers. The eggs hatch and the larvae tunnel inside. After several days, the workers head falls off, the fly larvae then pupate, complete their development and emerge to seek out new worker ants. The advantages of these parasites are their ability to move about to find potential victims and to be host specific. Species of these flies have been investigated for controlling fire ants in the southern U.S. (Porter, et al., 1995; Porter, et al., 1997), but little work has been done pertaining to their biology and use as a biocontrol for *Camponotus* (Fox, 1887; Disney, 1994). We examined the use of *A. horridus* for controlling *Camponotus vicinus* as part of our broader effort to better understand the biology of carpenter ants.

6.3: MATERIALS AND METHODS:

Carpenter ants in a colony of *C. vicinus* were collected during February and March 2001 in Benton Co., Oregon. Ants were found in small logs and semi-decayed pieces of wood and removed by breaking the wood open with chisels and a

hammer and shaking or brushing the ants out into plastic containers. Once in containers ants were taken to the lab and kept under refrigeration until needed. Ant workers were placed, in groups of 10, into petri dishes and fed glucose alone or amended with 3.33, 6.66, or 13.32 x10 $^{-4}$ mg/ml mixture of the fungicide propiconazole and the anti-biotic tetracycline. These tests were initially started as feeding studies, until the flies were detected in the workers. The ants received 100 µl of glucose or the mixtures every three days for two weeks. Each treatment was replicated on three petri dishes each containing 10 workers.

In addition, two other trials were established. The first used the same procedures described above but employed 20 workers per petri dish, while in the second, 40 workers were placed into large plastic containers and fed chemical treatments via a small diameter tube. Ants were monitored daily for four weeks. Ant heads usually began falling off within 10 days. The width of the decapitated ant heads was measured with an ocular micrometer. Heads were placed into petri dishes and incubated at 25° C and 70 % relative humidity (RH) to determine when adult flies emerged. We examined the effect of elevated temperatures on decapitation by exposing 120 worker ants from the parasitized colony at 39° C for 48 hours. We used this temperature because in a trail test we found that it did not affect the worker ants but had an effect on microbiota associated with the ants.

We also surveyed a total of eight *C. vicinus* colonies in the area for the presence of the parasitoid. Four were from McDonald-Dunn research forest and four were from Philomath, Oregon. Worker ants were removed from colonies as above and placed into plastic tubs. The ants were fed a high dosage (13.32 x10 $^{-4}$ mg/ml) of the propiconazole-tetracycline mixture via small tubes. Ants were fed for four weeks and the number of decapitated heads was recorded.

Finally, we placed emerging flies in chambers containing worker ants to determine if we could encourage them to mate and lay eggs on workers. Groups of 10 workers were placed into a plastic flight box (300 x 220 x 100 mm) along with emerging flies and the worker behavior was observed.

6.4: RESULTS AND DISCUSSION

Apocephallus horridus parasitized 1 to 15 % of carpenter ant workers in the colonies surveyed. Two colonies from MacDonald forest were parasitized (8 and 15%), and one from Philomath was parasitized (1%). While this suggests that the fly is not normally a major parasite of workers, elevated parasitism levels in some colonies imply that environmental conditions around colonies may make them more susceptible to parasitism. It also suggests that parasitism levels may be encouraged by either release of flies or manipulation of colony conditions.

The frequency of flies from workers fed glucose with or without propiconazole-tetracycline varied with dosage. In general, fly incidence increased with increased propiconazole-tetracycline dosage. Results of the larger trial produced similar results. While propiconazole-tetracycline should not markedly affect fly physiology, it may affect fungi associated with either the ants or the parasite (Figure 7.1a).

Laboratory trials using larger numbers of ants suggested that the presence of the fungicide produced less clear-cut effects on the incidence of decapitated ants. Low levels of fungicide were associated with a sharp increase in head decapitation, but levels dropped then increased at higher dosages (Figures 7.1c). The ambiguous results suggest that the biocides may have more subtle effects on fly/carpenter ant interactions.

Head width measurements of decapitated and normal worker heads suggested that decapitation increased with decreasing worker head width. The average head width of decapitated ants was 1.6 mm. The average head width of non-parasitized ants from the same colony was 1.8mm. Smaller workers may be less able to fend off flies attempting to oviposit around their heads. Workers in other species have been observed assuming defensive positions when flies appear (Feener and Brown, 1992).

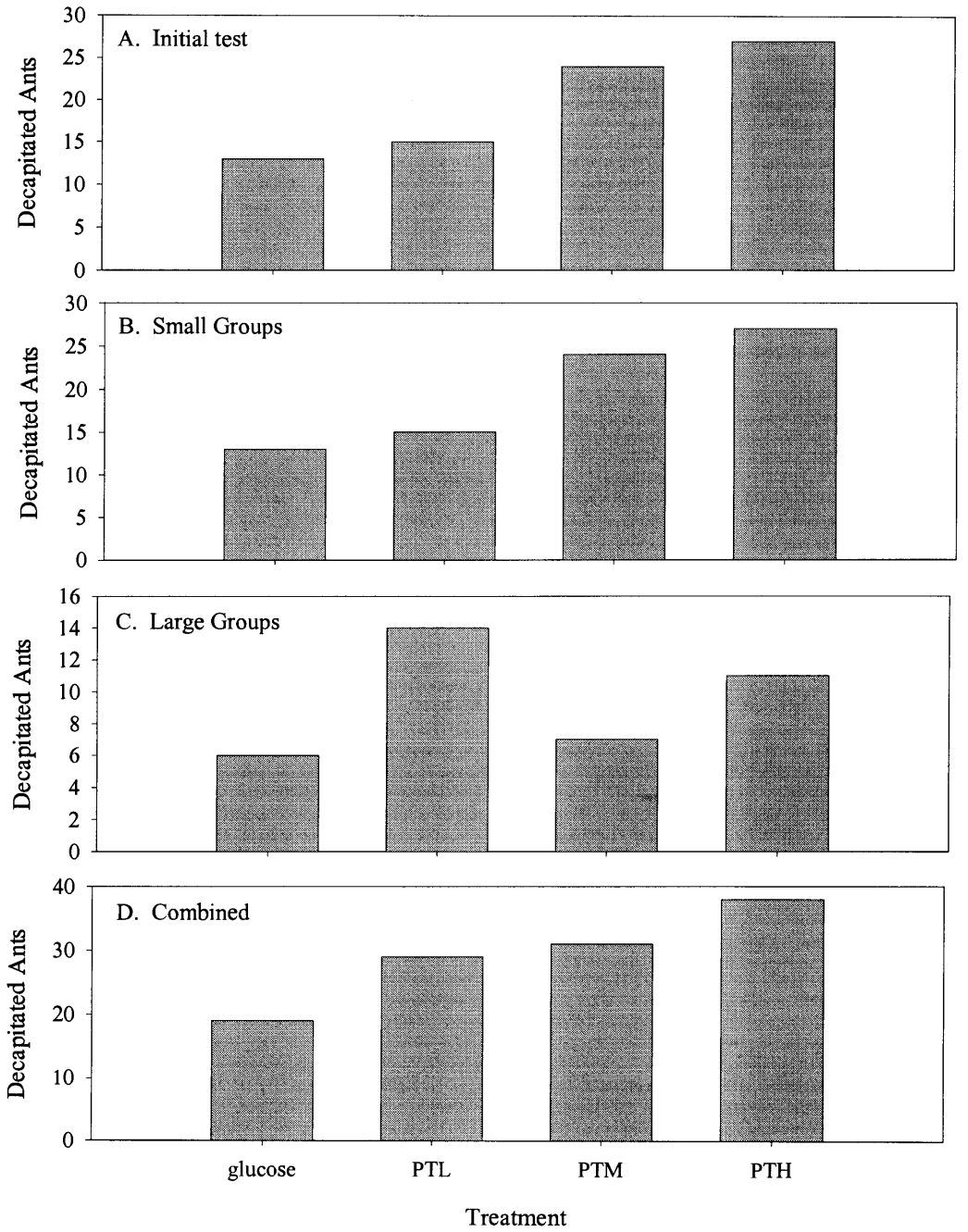

Figure 6.1: Number of decapitated ants after feeding *Camponotus vicinus* workers glucose, and low (PTL), medium (PTM), and high (PTH) dosages of propiconazole-tetracylcine mixed with glucose for A. Initial experiment, B. Small ant groups fed 100, C. Large ant groups, and D. small and large groups combined.

Exposure to elevated temperatures (39° C) for 2 days appeared to eliminate the incidence of decapitated ants. While 39° C is not extremely hot, most carpenter ant nests are located in materials that tend to be insulated from substantial heating. For example, even though wood pole surfaces reach elevated temperatures that can exceed 60° C, this heat is transmitted relatively slowly into the interior where carpenter ant galleries are located. The ability to alter parasite incidence by short-term heat exposure implies, however, that subtle environmental changes may have dramatic effects on successful parasitism. Understanding these effects will be essential as we further explore the potential for using this insect for carpenter ant control.

Attempts at mating flies and getting them to attack ants in the lab were unsuccessful, although ants sometimes attempted to fend off flies, the flies and ants kept separated from one another in the flight box. The ability to produce large quantities of flies would be essential for use of these insects in any biocontrol program. Clearly further studies will be necessary to determine conditions necessary for mating and oviposition.

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CHAPTER 7: CONCLUSIONS

The original goal of this dissertation research was to develop an improved understanding of carpenter ant biology. The survey of Oregon pest control operators aided our understanding of which wood destroying organisms were most prevalent in various regions of the state. It also gave us an idea of what conditions were conducive to attack by these organisms and highlighted some training needs. Future surveys should also examine PCOs in reference to the number of treatments and cost of those treatments so we can accurately gauge the economic effects of wood destroying organisms, including carpenter ants, in Oregon.

Most studies examining carpenter ants have examined foraging ecology and toxicology and paid little attention to moisture regimes required by these ants. Colony initiation and desiccation resistance studies showed that *C. vicinus* was able to survive longer and rear offspring at lower relative humidities and substrate moisture contents than *C. modoc*. Hansen (1985) found that *C. modoc* was more common in structures than *C. vicinus*, but that *C. vicinus* produced much larger colonies. The ability of this ant to survive in both mesic and xeric habitats in the west implies that it would be extremely common in households throughout Oregon. Information on the incidence of each species is lacking and it would be useful to perform a survey of pest ant species in Oregon and the northwest in general. The reasons for the ability of *C. vicinus* to withstand desiccation are unknown. The next step in this research is to discover why *C. vicinus* tolerates low moisture. The results of my tests with colony initiation in different substrates indicate that wood species can affect larval development. If so, it would be interesting to find out which wood chemicals, particularly in western redcedar, are detrimental to larval development. One important aspect of carpenter ant biology that was not examined was excavation of substrate. It may be possible to use this wood block apparatus to examine tunneling behavior of groups of worker ants in wood at varying moisture

contents. Because wood is easier to excavate as it gets wetter, it would be useful to determine if moisture thresholds exist for excavation.

The prospect that microorganisms associated with *Camponotus* might enhance ant biology opens new areas for controlling ants via their associates. Although previous studies found fungal hyphae in the digestive tracts of *Camponotus* (Ayre, 1963), yeast species in the buccal cavity of this ant species, including *Debaryomyces polymorphus*, indicate a more central association. The association of this yeast with other forest ants suggests that this yeast may play a more general role in nutrition. Further studies to better elucidate the benefits of this association may help develop more effective methods of prevention.

Studies using artificial diets and the effects of yeast on defaunated ants reveal that there is much to learn about ant nutrition. Other investigators have used molecular techniques to examine the relationships of arthropods with microorganisms and found that entire communities of commensal and facultative symibionts in arthropods contribute nutrients and enzymes (Kaufman, et al. 2000). Similar studies would help to better delineate ant-microbial associations.

It is important to note the yeasts are only one group of microorganisms associated with *Camponotus*. Hansen et al. (1999) found many types of bacteria associated with the infrabuccal pocket as well. The role of these microorganisms is assumed to be symbiotic, although their exact functions remain unknown.

Research in the area of artificial diets and nutrient studies in ants is far from complete. Little information exists on various nutrients in ants. Furthermore, there is only circumstantial evidence that diet completely affects caste determination in ants. Development of an optimal artificial diet would confirm this. Future experiments with diets and carpenter ants should also evaluate the effects of changes in diet on microorganisms in the gut tract of these ants. Since carpenter ants feed on a high carbohydrate diet in the early spring and then change to a higher protein diet by summer, it would be useful to determine how microorganism-ant associations change with diet. Diet or exposure to chemicals may also affect the susceptibility of ants to pathogens and parasitoids. For example we have some

evidence that exposure to some biocides can affect the ability of the parasitic fly *Apocephalus horridus* . Clearly, our results suggest that there is much to learn about the biology of carpenter ants. This knowledge could be used to determine when and where carpenter ants are most likely to occur. This information can then be used to develop more rational methods for prevention that reduce the dependence on traditional chemical remedies.

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APPENDIX

APPENDIX: SURVEY TO ASSESS PRESENCE OF INSECT AND FUNGAL ATTACK IN STRUCTURES.

Survey to Assess Presence of Insect and Fungal Attack in Structures

Location _____County_____City_____Zip_____

1. Structure:

Location: Rural Residential/suburban City

Construction:

Full Basement Concrete Pad Half Basement
CrawlSpace Other(specify):_____

Check all that were found:

Fungi Carpenter Ants
Dampwood Termites Wood Boring Beetles
Subterranean Termites

Conditions Favoring WDOs:

Cellulose Debris Earth-wood contact Excessive moisture
Insufficient ventilation Faulty grades Dirt Fill
Inadequate clearance Other:_____

Miscellaneous

Sub-floor foil barrier Plumbing Leak
Vapor barrier Inaccessible areas
Sub-floor Insulated Further inspec. rec

WDO
Sills/Joists/Subfloor_____

Flooring/Walls/Interior trim_____

Exterior Doors, windows
and frames _____

Furniture_____

WDO
Roof rafters and joists_____

Roof trim /Siding_____

Exterior columns,
steps,porches_____