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ABSTRACT

Tunnel patterns of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, and its foraging behavior relative to the non-repellent wood preservative, disodium octaborate tetrahydrate (DOT), were studied in laboratory and field conditions using two-dimensional foraging arenas with multiple foraging sites. The initial frequency of tunnels entering arenas was uniformly distributed but became significantly skewed towards foraging sites farther out. This pattern occurred when wood was present and absent, suggesting that tunneling was affected by the physical aspects of discovered sites and not the presence of a food resource. Additional tests revealed that sites with open space elicited significantly more tunnels with a greater dispersion of tunnels outward from the point of site discovery than sites with solid food or non-food objects.

Tunnel width and segmentation were found to be significantly correlated to a foraging group’s average worker size. Groups of larger workers constructed less segmented galleries with wider tunnels and were more efficient at discovering food items. Groups of smaller sized workers exhibited a slower rate of discovery but found the same amount of food items over time.

Discrete group of foragers from two different colonies showed no differences in amount of tunneling and wood consumption under controlled laboratory conditions; however, significant differences in tunneling and feeding were found when comparing the same two colonies in the field. Population estimates suggested that colony size could have been a factor. Observations on tunneling in the field over a three-day period revealed that tunneling might be related to air temperature with a lag effect.
The presence of treated wood to manufacturer recommended retentions of DOT did not deter termite tunneling in the surrounding substrate. Foragers continued to taste treated wood as it was moved to new locations, and they fed on untreated wood in locations previously occupied by treated wood. Foragers that ingested lethal amounts of borate from treated wood did not remain and die in the vicinity of the treated wood. Avoidance was more related to the location of treated wood than to recognition of the treatment. The potential role of pheromones in discretionary feeding is discussed.
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CHAPTER 1

Introduction

Subterranean termites are among the most economically important insect pests in the United States. Their predilection for lumber used in construction generates expenses totaling approximately $1.5 billion each year for their control (Su and Scheffrahn 2000). One of the most destructive species of termites in certain areas of the United States is the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). When compared to other subterranean termites, *C. formosanus* colonies are typically larger, consume wood at greater rates, and have the ability to form aerial nests (Delaplane 1991). Native to China (Kistner 1985), the Formosan subterranean termite was first reported in Hawaii in the early 1900’s (Swezey 1914). Subsequent accidental introductions to the mainland United States resulted in its discovery in Louisiana, Texas and South Carolina in the 1960’s (King and Spink 1969), Alabama, Mississippi, and Florida during the 1980’s (Woodson et al. 2001), and in San Diego, California in 1992 (Atkinson et al 1993). From each of these footholds it has spread and continues to expand its distribution as environmental constraints allow.

Eliminating infestations of *C. formosanus* can be problematic due to difficulties associated with their social organization, aggressive foraging habits, and cryptic underground lifestyle. Like all subterranean termites they tunnel unseen through the earth in search of food and often feed undetected on lumber until structural failure occurs. Conventional methods of preventing subterranean termite attack have long been centered on the implementation of chemical barriers – substances applied to the soil that denied passage to foraging termites (Jones 1990a). Certain shortcomings of this approach,
however, led researchers to explore the use of termiticides that, instead of simply repelling termites, took advantage of the same characteristics that make subterranean termites so difficult to control (Su et al. 1984).

Termiticides were classified into three types according to how foraging termites reacted to them. Type I termiticides were defined as those chemicals that were repellent to termites and elicited an immediate avoidance by foragers; the type II classification was given to termiticides that were initially non-repellent, but killed termites quickly, resulting in a repellent area of dead foragers at the treatment sites. The final group, type III termiticides, were classified as chemicals that were non-repellent, but possessed a delayed or slow mode of action, and therefore created a treated area that was unassociated with mortality as the poisoned individuals moved away, and was consequently undetectable to subsequent foragers (Su et al. 1982). Type III termiticides are generally either insect growth regulators or some other type of slow acting toxicant. These compounds have received much attention in recent years because of their usefulness in baiting systems and liquid soil applications that, through exploiting social behaviors of subterranean termites such as trophallaxis and grooming, have the potential to kill a large majority of a colony’s foraging population, if not eradicate the entire colony (Su et al. 1995a).

The capacity of a type III termiticide to significantly impact a colony of subterranean termites is dependent upon its ability to be brought into contact with the colony’s foragers. The resulting uptake of the active ingredient into the foraging population is achieved in a variety of ways dependent upon the strategy employed by the termiticide product being used. A baiting system, for example, relies on discovery of the
bait and the subsequent unknowing ingestion of the active ingredient through feeding (Su et al. 1995b). To be effective in a timely manner, bait stations must be applied to the soil directly over active tunnel systems, or in areas where future-tunneling activity is anticipated. Unfortunately, even though termites have been referred to as the earth’s first architects (Marais 1937), they never provide blueprints of their projects. In the absence of visible above ground clues, pest management professionals are typically left to speculation as to the location and direction of their underground tunnel systems. Without knowing where tunnels are, the easiest method and the one most routinely employed by pest management companies, is to install bait stations at fixed intervals around the entire perimeter of the infested structure (Jones 2003). Unfortunately, this approach can result in a lengthy and drawn out period from system installation to the ultimate goal of eliminating the infestation (Potter 1997). To increase the speed of baiting efficacy, some researchers have advocated the use of a more targeted approach, with increased emphasis on placement of bait stations in areas conducive to termite activity. Studies in the field have suggested that bait stations installed in locations with evidence of termite activity and features promoting high moisture levels show a greater likelihood of discovery by *Reticulitermes* spp. in comparison to stations located around a structure in a uniform pattern (Jones 2003, Henderson et al. 1998). A more precise targeted approach has the potential to avoid prolonged baiting treatments, but requires a comprehensive understanding of termite behavior.

Recent studies have provided some insights on conditions that are conducive to subterranean termite foraging and have enhanced our ability to predict likely locations of tunneling activity. Experimentation with two-dimensional foraging arenas has supported
these findings by determining that moisture in the tunneling substrate stimulates tunneling by *Coptotermes* spp. (Su and Puche 2003, Evans 2003). Additionally, termites may also be attracted to areas that contain chemical exudates from food resources. Cornelius et al. (2002) found that *C. formosanus* Shiraki explored sand that was treated with methanol extracts of fungus-infected sawdust significantly more than sand treated with extracts from uninfected sawdust. Others factors such as loose soil, the presence of guides or linear objects, and existing passages or gaps also have been shown to stimulate tunneling behavior (Tucker et al. 2004, Pitts-Singer and Forschler 2000).

This dissertation presents further work on the tunneling behavior of the Formosan subterranean termite and the elements of the underground environment that influence its ability to find food. Presented here are the results of foraging experiments that shed light on the sequence of events that occur as termites pioneer new foraging territory and discover new food resources. The question of whether tunneling is random or systematic is addressed, and the effect of a colony’s average worker size on its tunneling ability is examined. Response of Formosan subterranean termites to physical objects in the soil is investigated and tunneling is compared between laboratory and field conditions.

Foraging behavior in response to the inorganic waterborne wood preservative, disodium octaborate tetrahydrate (DOT) is also examined. Pressure treatment with borates is an increasingly popular method to protect lumber and wood products used in interior construction from subterranean termites. When compared to some of the alternative waterborne chemical preservatives that are toxic to termites, including chromated copper arsenate (CCA), a popular arsenical compound that has recently come under scrutiny of the United States EPA, borates have many benefits. They exhibit low
mammalian toxicity, leave no odor, stain, and are able to penetrate into the heartwood of wood species, such as Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), that is otherwise resistant to preservative penetration (Grace et al. 1992). For these reasons, there is increasing interest in use of borate compounds for protecting wood, and how the use of borates affects foraging by subterranean termites.
CHAPTER 2.
Tunnel Orientation and Search Pattern Sequence of the Formosan Subterranean Termite

2.1 Introduction

Over the past decade the rationale of subterranean termite control with repellent chemical barriers has come under scrutiny, and an alternative paradigm of population suppression has emerged. This approach to termite control relies on nonrepellent baits or insecticide treatments that do not stimulate behavioral defenses or avoidance. With the advent of these nonrepellent treatments, an understanding of termite foraging and tunneling behavior in the subsurface soil environment has become increasingly important. Much of the knowledge of termite foraging behavior has been gained through either direct gallery excavation (Ratcliffe and Greaves 1940, Greaves 1962, King and Spink 1969) or studies on baited plots by using spatio-temporal and mark-release-recapture methods (LaPage et al. 1973, Su et al. 1984, Grace et al. 1989, Jones 1990b). Although these methods have provided valuable insights into the ecology and behavior of subterranean termites, they offer no direct information on tunnel formation during territory expansion.

Several laboratory studies using two-dimensional surfaces have added to our understanding of foraging behavior by creating an artificial window into the cryptic subterranean environment of termites. Using two large vertical Plexiglas sheets to create a two-dimensional tunneling environment, Robson et al. (1995) described the colony-level search pattern of Reticulitermes flavipes (Kollar) as nonrandom, suggesting that in the absence of resource cues exploratory tunnels divide a search area evenly to minimize
redundancy. Reinhard et al. (1997) conducted similar studies with *Reticulitermes santonensis* De Feytaud and found that foragers constructed a "regularly branched net" of trails that systematically covered all directions of the territory in the absence of wood cues. Arena tunneling studies conducted on the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, have shown that available food size influences the search tunnel network created by foragers in amount of branching and length of tunnels (Hedlund and Henderson 1999).

I further explore *C. formosanus* tunneling behavior in the present study. Spatial analysis of digital images was used to elucidate tunneling as foragers pioneer a new area of substrate containing multiple foraging sites, and to compare tunneling in the presence and absence of food resources.

### 2.2 Materials and Methods

#### 2.2.1 Arena Design.

Foraging trials were conducted in laboratory arenas consisting of three layers (85 by 85 cm) of clear acrylic (2.5 mm in thickness) with the middle layer cut as a frame (5 cm in width) to create a space (75 by 75 by 0.25 cm) between the two outer layers (Fig. 2.1). Silica sand (40-100 mesh, Fisher Scientific, Pittsburgh, PA) and distilled water (approximately 18% by weight) were added to the middle space to serve as the foraging substrate. The top layer of the arena had holes (3.1 cm in diameter) drilled every 20 cm in a grid pattern with one large hole (8.2 cm in diameter) drilled in the center. Plastic vials sized for press fitting into the 3.1 cm diameter holes (capacity 48.1 ml) and with the bottoms removed were inserted into the smaller holes to serve as foraging sites. These
sites were either left empty or contained wafers (2.5 by 2.0 by 0.5 cm) of Douglas fir, *Pseudotsuga menziessii* (Mirb.) Franco. A plastic jar (capacity 500 ml) with the bottom removed was press fitted into the large center hole. This center site was the termites’ point of entry into the arena, or initiation site, where termites were added and began tunneling. The initiation site contained two moistened, 9-cm-diameter filter paper discs to provide both a substrate and food for the termites. The entire arena was kept in a dark room and backlit from beneath with fluorescent lighting so that digital photographs of the tunnels could be taken from above for computer analysis.

### 2.2.2 Protocol.

Termites were collected from four different field sites and are referred to here as colonies A, B, C, and D. Colonies A (Miller Hall), B (Gilmore Hall), and D (Kuykendall Annex) are located on the campus of the University of Hawaii at Manoa. Colony C is found on the windward side of Oahu at the University of Hawaii agricultural experiment station in Waimanalo. Two arenas were used for each colony, with one arena containing Douglas fir wafers in each foraging site and the other arena containing only empty foraging sites. Fifteen hundred termites (90% workers and 10% soldiers) were added to each arena. Tunneling in the arenas was monitored for a period of 48 h. Digital photographs (D-600L digital camera, Olympus, Melville, NY) were taken every 3 h for the first 24 h to distinguish between entry and exit tunnels at the four foraging sites closest to the initiation site. During the second 24 h, as tunneling proceeded outward from the center region of the arena, photographs were taken every 6 h. The arenas were housed in a facility with limited temperature control; therefore, temperature of the indoor ambient environment fluctuated concurrently with the outside daily temperature. Indoor
Figure 2.1 Arena Design and Set-up in the Laboratory. Fastening together two layers of clear acrylic forms the tunneling arena. Wet sand between the layers serves as the tunneling substrate through which termites must dig to find food after they are added to the center jar.
temperatures reached a mean high of 26°C in the late afternoon and a mean low of 22°C in the early morning.

2.2.3 Analysis.

ArcView 3.2 GIS software (ESRI Inc., Redlands, CA) was used to process images and digitize tunnels. Tunnels were classified relative to the site they originated from. Tunnel classification allowed the sequence of tunnel development and any patterns leading to site discovery to be followed. A site was classified as primary, secondary, or tertiary according to the origin of the tunnel that first intercepted it. For example, a site intercepted by a tunnel that originated from the initiation site is termed primary, and a site intercepted by a tunnel that originated from a primary site is termed secondary. To examine the distribution and orientation of tunnels leaving the initiation site and reaching the four nearest foraging sites, the 48-h photograph was overlaid with circles marking the perimeter of the initiation site and the perimeter where the first four foraging sites could be encountered. The circles were divided into 20 slices with each slice occupying an arc on the circle perimeter equal to the span of an individual foraging site. The slices were then grouped into five sectors, such that each sector contained slices from four opposing directions in the circle (Fig. 2.2). The intersection of tunnels crossing both perimeters were marked in each sector and totaled (Table 1). Chi-square analysis (SPSS for Windows 9.0; SPSS Inc., Chicago, IL) was used to compare the frequency of tunnels in each sector.
Figure 2.2 Points where tunnels exit the perimeter of the initiation site and cross the perimeter of the first four foraging sites. The two perimeters are divided into 20 slices that comprise five sectors. Slices of the same number constitute one sector.
Table 2.1 Chi square values and probabilities for the distribution of tunnels in the five sectors from all colonies in both treatments.

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<th>Colony</th>
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<th>Wood absent</th>
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<td></td>
<td>Inner perimeter</td>
<td>Outer perimeter</td>
</tr>
<tr>
<td></td>
<td>Sectors</td>
<td>Sectors</td>
</tr>
<tr>
<td></td>
<td>1 2 3 4 5</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>Colony A</td>
<td>4 0 1 2 0</td>
<td>4 0 1 2 0</td>
</tr>
<tr>
<td>Colony B</td>
<td>1 3 4 4 2</td>
<td>4 2 1 2 0</td>
</tr>
<tr>
<td>Colony C</td>
<td>4 1 3 3 2</td>
<td>5 1 1 2 0</td>
</tr>
<tr>
<td>Colony D</td>
<td>3 4 2 2 4</td>
<td>8 1 2 1 0</td>
</tr>
<tr>
<td>Obs. Total</td>
<td>12.0 8.0 10.0 11.0 8.0</td>
<td>21.0 4.0 5.0 7.0 0.0</td>
</tr>
<tr>
<td>Exp. Total$^1$</td>
<td>9.8 9.8 9.8 9.8 9.8</td>
<td>7.4 7.4 7.4 7.4 7.4</td>
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<tr>
<td>Chi Square value</td>
<td>1.31</td>
<td>34.76</td>
</tr>
<tr>
<td>p value</td>
<td>0.86</td>
<td>&lt;.001</td>
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$^1$ Under the hypothesis of a uniform circular or "random" distribution.
2.3 Results

Figure 2.3 illustrates through a series of digitized snapshots the general pattern of events observed over 48 h as the termites tunneled outward from the initiation site in an arena. These events are summarized as follows: 9 h, foragers begin tunneling outward from the initiation site, with tunnels emanating out in a pattern similar to the spokes of a wheel (not all initial tunnels are begun at the same time nor proceed at the same rate); 15 h, when a tunnel intercepts a foraging site, foragers are recruited to that area and tunneling increases in the localized area of the site and decreases in areas where foraging sites have not been encountered; 30 h, upon recruitment to a discovered site, foragers create new search tunnels, which radiate out in the wheel-spoke pattern (most of these search tunnels are abandoned if nothing in the vicinity of the new site is discovered, but one or two main tunnels will continue out in the general direction from where they originated); 48 h, this pattern is repeated upon contact with every new site, when tunnels reach the boundaries of the arena they follow the edge with little deviation, and the majority of tunneling activity continues along these edges until the arena is circumnavigated; and 4 wk, daily tunneling activity decreases after the first few days, but eventually foraging termites construct tunnels into the remaining vacant areas of the arena until the majority of the substrate is explored. The final tunnel pattern after 48 h for all colonies in both treatments is shown in Fig. 4. No observable differences were evident between colonies or within colonies by treatment.

Points where tunnels crossed the initiation site perimeter and the perimeter at which foraging sites could be encountered were pooled from all colonies according to treatment (Fig. 1). Assuming that tunnels show no directional preference, the expected
Figure 2.3 Tunneling sequence of colony D (wood absent) that was representative of the general pattern observed in all colonies (4 wks is actual photograph of arena). Sites are classified as primary, secondary, or tertiary according to the origin of the tunnel that first intercepted it (i.e. a site intercepted by a tunnel that originated from the initiation site is termed primary, and a site intercepted by a tunnel that originated from a primary site is termed secondary).
Figure 2.4 Digitized tunnel patterns of all colonies and treatments.
frequency of tunnel crossing points per sector, defined as \( \Sigma n/k \) where \( n \) = number of points and \( k \) = the number of sectors, should be equal (Batschelet 1965). The inner perimeter tunnel distributions in arenas with and without wood were not significantly different \( (\chi^2 = 1.31, df = 4, P = 0.86, \) and \( \chi^2 = 2.36, df = 4, P = 0.67, \) respectively). Tunnel distribution was significantly affected by the presence of foraging sites in arenas with and without wood \( (\chi^2 = 34.76, df = 4, P < 0.001, \) and \( \chi^2 = 31.75, df = 4, P < 0.001, \) respectively). Inspection of frequencies at the outer perimeters indicated how the tunnel distribution was affected. Sector 1, the sector containing foraging sites, had three to four times more tunnels than the other sectors (Table 1).

2.4 Discussion

My observations on the sequence of tunneling events by groups of foragers support the findings of Robson et al. (1995), working with R. flavipes, that subterranean termites show a significant tendency to tunnel in directions that divide their search area evenly. In my study, it appears that after the initial tunnel is begun by a group of foragers, subsequent tunnels are started that extend outward from the initiation site to unexplored areas. The resulting pattern of tunnels from the initiation site is radially symmetrical.

When all trials were pooled by treatment, the results of the chi-square test indicated that initially, with or without wood in the foraging sites, tunnels displayed no orientation towards foraging sites. These initial tunnels entering the arena displayed a uniform circular distribution. In other words, each possible tunnel entry point had the same probability of being chosen by termites. In circular statistics, this distribution is
considered random (Batschelet 1965). In this context the word “random” is used to mean nondirectional, and refers to the alignment of a pooled group of tunnels from multiple foraging bouts relative to the compass points of the foraging arena. Initial nondirectional searching also has been described in laboratory tests on Constrictotermes cyphergaster (Silvestri), a common tree-nesting termitid in Central Brazil (Souto et al. 1999).

Approximately 10 cm from the initiation site, I found, however, that tunnels deviated significantly from this nondirectional pattern. This difference consisted of more tunnels being aligned toward the foraging sites. It is not entirely clear why more tunnels intercepted the foraging sites, but my observations suggest that once a foraging site is discovered, tunneling activity increases in that general area and decreases or ceases in other areas where termites have not encountered a site. Therefore, some tunnels outside of the zones containing foraging sites were abandoned before reaching the foraging site perimeter. This explanation is supported by the observation that once foragers discovered a site, recruitment to that site occurred and a new search pattern was begun from that site. This systematic series of events created an apparent orientation towards sites and a nonrandom pattern in the resulting experimental gallery system.

Reinhard et al. (1997) attributed systematic search by termites in a vermiculite substrate and in open air to perceived volatiles emanating from wood. Earlier work referenced by Clement et al. (1988) showed that terpenes at low concentrations from Pinus wood can act as kairomones and guide subterranean termites in their tunneling search for food. It is possible, however, that some subterranean environments may not contain sufficient chemical attractants or allow circulation of chemical cues to the extent that they diffuse and are made available in a loose substrate or open-air setting. Densely
packed substrates may inhibit the formation of long distance chemical gradients leading to food resources and could require that foraging subterranean termites rely more on tunneling strategies and physical cues as they pioneer new areas in search of food. For example, thermal gradients have been shown to serve as food detection cues in mesic habitats for two species of desert subterranean termites (Ettershank et al. 1980). The shift to a reliance on tactile stimuli in the absence of chemical cues is also made by the army ant *Neivamyrmex nigrescens* (Cresson) (Topoff and Lawson 1979).

The systematic pattern of tunneling that I observed was present regardless of the presence or absence of wood in the foraging sites, suggesting that the directional effect was created by the physical presence of the sites (as anomalies in the tunneling substrate) and not by the presence of food. Thus, physical heterogeneity alone in the substrate can affect the direction of tunneling by foragers in search of new food resources. The role of non-chemical topographic features in pioneering or primary foraging in an open air environment has been documented in some ant and termite species (Klotz and Reid 1992, Jander and Daumer 1974), but little is known about their effect in a subterranean setting.

In addition to a positive tunneling response to the foraging sites, I observed that upon encounter with the edge of the arena, *C. formosanus* concentrated tunneling at the edge until the arena was circumnavigated. It is uncertain, however, whether foragers associate a substrate anomaly with food; or if the anomaly simply makes tunneling easier. In this case the hollow spaces could have provided more area for tunnel initiation and free volume for substrate deposition, and the firm surface of the edge possibly facilitated tunnel excavation. The carpenter ant, *Camponotus pennsylvanicus* (DeGeer), uses physical components of its environment such as structural guidelines to increase
"locomotory efficiency" and thereby saves on time and energy in foraging (Klotz and Reid 2000).

An additional explanation for this sequence of tunnel patterns is that it is possibly an adaptation for locating food resources with a clumped distribution. That is, when foragers find one site, they expect more to be in the vicinity. Williams (1977) cited exploratory “runways” constructed in the immediate area of a discovered food source by several species of subterranean termites and attributed this to adaptation to an environment with discontinuous distribution of food resources. Field studies testing the systematic discovery of foraging sites, however, are rare and inconclusive. Site discovery, with respect to distance between sites (1.5 m), by the desert subterranean termite, *Heterotermes aureus* (Snyder), was determined to be a random process (Jones et al. 1987). My study suggests that systematic discovery can occur, but at site intervals much smaller than are typically tested in the field. In terms of commercial application, the systematic pattern I observed also lends support for the use of auxiliary baiting stations around an active baiting site. It should be noted however, that the majority of search tunnels around a newly discovered site were usually abandoned after no further sites in the immediate vicinity were found, suggesting that timing of placement from the active site may be an important factor in the use of auxiliary stations.

I am mindful that the issue of scale can be a troubling element when arriving at conclusions through laboratory foraging studies. Actual foraging territories of *C. formosanus* can extend 100 m and involve millions of foragers (Su and Scheffrahn 1988b). In this study I dealt with an area of 0.5625 m² and only a small fraction of a functional colony's foraging population. The cryptic habits of termite populations make
field observations of behavior quite challenging. However, to further substantiate my laboratory findings, I am currently exploring methods to direct termite foragers from field colonies into arenas that will permit observation.
CHAPTER 3

Effect of Average Worker Size on Tunneling Behavior of Formosan Subterranean Termite Colonies

3.1 Introduction

Colonies of subterranean termites can display considerable variation in average worker size. Mean worker wet weights of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, have been reported from 2.05 mg (Cornelius and Osbrink 2001) to 6.81 mg (Grace et al. 1995). Differences in average worker size can occur between colonies in the same general habitat (Su and La Fage 1984a), between colonies utilizing different hosts in a natural habitat (Waller 1988), between colonies in natural and urban habitats (Haagsma and Rust 1995), in the same colony according to season (Waller and LaFage 1987), or in the same colony according to colony age (Grace et al. 1995).

Research on the division of labor in subterranean termite colonies has shown that large workers are more efficient at tunneling than smaller sized workers. Observing marked individuals of *Reticulitermes santonensis* De Feytaud, Garnier-Sillam (1983) found a link between the size or “stage” of a worker and digging activity, with older, larger stages digging more and at faster rates than younger, smaller stages. Similarly, Crosland et al. (1997) showed that large workers of *Reticulitermes fukienensis* Light tunneled approximately 10 times the distance of smaller sized workers. Having groups of smaller sized workers perform various foraging behaviors in the presence and absence of larger workers, it was further found that tunneling was the only behavior smaller workers could not adequately compensate for in the absence larger workers. This finding
prompted speculation that perhaps smaller workers utilize a different foraging pattern than larger workers to make up for their tunneling inefficiency due to size (Crosland and Traniello 1997).

The current study investigates the effects of a foraging population’s average worker size on its tunneling behavior. I explore this relationship in two-dimensional foraging arenas using large samples of termites from four different C. formosanus colonies covering a wide range of average worker size, with the primary objective to determine if colonies with larger workers are more efficient at foraging than those with smaller workers.

3.2 Materials and Methods

3.2.1 Arena Design.

Foraging trials were conducted in the laboratory using two-dimensional arenas as described in the previous chapter. The arenas were designed such that two layers (85 by 85 by 0.25 cm and 85 by 85 by 0.50 cm) of clear acrylic were fastened together with metal screws to create a space (75 by 75 by 0.25 cm) in the middle (Fig. 2.1). Eight small squares of acrylic with screw holes were affixed to the bottom layer to keep the space uniform after assembly. Silica sand [40-100 mesh (150-425 μm sieve), Fisher Scientific, Pittsburgh, PA] and distilled water (approximately 18% by weight) were added to the middle space to serve as the foraging substrate. The top layer of the arena had holes (3.1 cm in diameter) drilled every 20 cm in a grid pattern with one large hole (8.2 cm in diameter) drilled in the center. Plastic vials (capacity 48 ml) with the bottoms removed were inserted into the smaller holes to serve as foraging sites. Foraging sites
contained pre-weighed wafers (2.5 by 2.0 by 0.5 cm) of Douglas fir, *Pseudotsuga menziessii* (Mirb.) Franco. A plastic jar (capacity 500 ml) with the bottom removed was placed in the large center hole. This center site was where the termites were added and into the arena and served as their point of entry into the tunneling substrate. Two 9 cm diameter discs of moistened filter paper were placed in the entry jar to provide surface area and an initial food source for the termites. Arenas rested on a metal grate supported by glass jars and were kept in a dark room. Backlighting from beneath arenas was achieved with florescent lighting, thus enabling tunnels to be photographed for computer analysis.

**3.2.2 Protocol.**

Termites were collected from four different field sites and are referred to here (according to average worker size from largest to smallest) as colonies I, II, III, and IV. Colony I was located in the central plateau region of Oahu at the Hawaii agricultural experiment station in Poamoho. Colony II was located on the University of Hawaii campus near Miller Hall. Colony III was located on the University of Hawaii campus near Gilmore Hall. Colony IV was located on the windward side of Oahu at a private residence in Kailua. There were a total of five foraging trials with one arena per colony in each trial. Treatment of the Kailua residence with a baiting system prevented colony IV from being used in the final trial. Termites were extracted from their colonies, counted, weighed, and added to arenas within 24 hours. Fifteen hundred termites (90% workers and 10% soldiers) were added to each arena. Average worker biomass for each colony was determined by weighing 5 groups of 50 workers. Each foraging trial was conducted over a 22-day period. Digital photographs (D-600L Digital Camera, OLYMPUS,
Melville, NY) were taken daily to monitor tunneling. At the end of each trial surviving termites were extracted, and the workers and soldiers were counted and preserved in 70% ethanol. All wood in the foraging sites and remaining filter paper in the initiation site were extracted, oven dried, and weighed. After all trials had been completed, 10 workers each were chosen at random from the preserved samples of termites for measurement of their head width. Heads were measured at the capsule’s widest point, and an average width was obtained for each colony in four of the five tunneling trials (specimens were not available from the final trial). The arenas were housed in a facility with limited temperature control; therefore, temperature of the ambient environment fluctuated concurrently with the outside daily temperature. Temperatures inside the facility ranged from highs of 26 to 30°C in the late afternoon to lows of 20 to 24°C in the early morning.

3.2.3 Tunneling and Foraging Analysis.

ArcView 3.2 GIS software (ESRI Inc., Redlands, CA) was used to process images and digitize tunnels for volume, length, and width measurements. To calculate tunnel volume I adopted the method of Hedlund and Henderson (1999) and used a depth constant of 0.2 cm because I similarly observed that tunnels either were constructed along the top or bottom surface of the arena with a thin layer of sand on the other side. All tunnel segments within each gallery system were counted after the 22-day foraging period. Foraging sites were inspected at the end of each trial and sites discovered by termites were counted. Total consumption (wood and filter paper) per termite was calculated under the assumption of linear mortality using the equation \( 2(W_1 - W_2)/(1350 + Nt) \) (Su and La Fage 1984b), where \( W_1 \) is the initial food weight, \( W_2 \) is the final food weight.
weight, and \( N_t \) is the number of workers alive upon extraction at 22 days. I excluded biomass from the equation because of my interest in examining average worker wet weight as a predictive variable.

### 3.2.4 Statistical Analysis.

The effects of colony source and date of foraging trial on worker wet weight, tunneling, foraging, and survivorship were examined by independent one way ANOVA tests using the general linear model procedure (Proc GLM) (SAS Institute 1999). Significant differences between colonies or trials were explored using the Tukey highly significant difference (HSD) test with an alpha level of 0.05. Regression analysis (Proc REG) (SAS Institute 1999) was used to evaluate the relationship between average worker wet weight and the experimental variables measured. Using the same procedure, the average worker wet weight of each colony was regressed against average head width, and the number of sites discovered on the first day of the trials was regressed against tunnel length.

### 3.3 Results

Comparison of mean worker wet weights separated the colonies into two general size classes, with Colonies I and II containing significantly larger workers than colonies III and IV \((F = 33.47, \text{df} = 3, 15; P < 0.0001)\) (Table 3.1). Regression of worker weight on head capsule width confirmed that heavier workers had wider heads (Fig. 3.1). There were significant differences between colonies in total volume tunneled \((F = 26.26; \text{df} = 3, 15; P < 0.0001)\), total length tunneled \((F = 24.25, \text{df} = 3, 15; P < 0.0001)\), tunnel width \((F = 33.92, \text{df} = 3, 15; P < 0.0001)\), tunnel segmentation \((F = 32.95, \text{df} = 3, 15; P < \)
0.0001), number of foraging sites discovered ($F = 11.36, df = 3, 15; P = 0.0004$), and survival ($F = 21.03, df = 3, 15; P < 0.0001$). There was no difference in consumption between the four colonies ($F = 1.52, df = 3, 15; P = 0.2502$). Colony I accounted for most of the variation, tunneling significantly less in terms of volume and length, creating the fewest segments, and discovering fewer foraging sites than the other three colonies. This was likely due to the fact that it was also significantly weaker, displaying on average 61 % survival compared to approximately 88 % survival of the other colonies.

Discounting colony I due to high mortality, colonies II, III, and IV differed only in tunnel width and segmentation. Colony II dug wider tunnels with fewer segments than colonies III and IV. The date on which foraging trials were conducted was found to have no significant effect on any of the experimental variables.

Regression of average worker weight on tunnel volume, tunnel length, tunnel width, number of tunnel segments, number of sites discovered, and consumption was conducted without colony I because of its higher mortality and consisted only of data from colonies II, III, and IV. The only variables to show a significant relationship to worker weight were tunnel width and number of tunnel segments. Average worker weight was negatively correlated to the number of tunnel segments (Fig. 3.2a) and positively correlated to tunnel width (Fig. 3.2b).

The average length tunneled by colonies II, III, and IV on each day of the foraging trials is represented in Fig. 3.3. I used tunnel length for this analysis instead of volume to correct for differences in tunnel width between the colonies.
### Table 3.1. Comparison of worker biomass, tunnel volume, tunnel length, tunnel width, number of tunnel segments, number of sites discovered, amount of wood consumed, and survival by colony

<table>
<thead>
<tr>
<th>Colony</th>
<th>Worker Wet Weight (mg)</th>
<th>Tunnel Volume (cm$^3$)</th>
<th>Tunnel Length (cm)</th>
<th>Tunnel Width (cm)</th>
<th>No. Tunnel Segments</th>
<th>No. Sites Discovered</th>
<th>Wood Consumption (mg/termite)</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poamoho (I)</td>
<td>5.22 ± 0.230a</td>
<td>54.74 ± 3.166a</td>
<td>618.00 ± 36.802a</td>
<td>0.484 ± 0.018a</td>
<td>55.6 ± 2.909a</td>
<td>7.6 ± 0.245a</td>
<td>3.69 ± 0.380a</td>
<td>61.13 ± 5.390a</td>
</tr>
<tr>
<td>Miller (II)</td>
<td>4.97 ± 0.210a</td>
<td>111.57 ± 4.334b</td>
<td>1003.74 ± 35.253b</td>
<td>0.682 ± 0.023b</td>
<td>112.6 ± 9.207b</td>
<td>11.2 ± 0.860b</td>
<td>3.84 ± 0.149a</td>
<td>88.97 ± 0.271b</td>
</tr>
<tr>
<td>Gilmore (III)</td>
<td>3.68 ± 0.132b</td>
<td>107.65 ± 7.909b</td>
<td>1140.75 ± 65.784b</td>
<td>0.452 ± 0.014a</td>
<td>158.4 ± 13.666c</td>
<td>12.0 ± 0.548b</td>
<td>3.32 ± 0.260a</td>
<td>88.81 ± 1.177b</td>
</tr>
<tr>
<td>Kent's (IV)</td>
<td>2.93 ± 0.087b</td>
<td>100.61 ± 3.452b</td>
<td>1067.61 ± 47.934b</td>
<td>0.474 ± 0.020a</td>
<td>169.0 ± 3.629c</td>
<td>12.8 ± 0.946b</td>
<td>3.04 ± 0.297a</td>
<td>87.18 ± 0.952b</td>
</tr>
</tbody>
</table>

Values are means (± SEM) of five replicates, except for colony IV that had four replicates. Means followed by the same letter within a row are not significantly different ($P > 0.05$, PROC GLM, Tukey-Kramer HSD test [SAS Institute 1999]).

- $a$Tunnel volume was obtained independently of length and width by calculating tunnel surface area and multiplying the resulting value by a depth constant.
- $b$Tunnel length was determined by running centerlines through all tunnel segments and calculating their distance.
- $c$Tunnel width was determined by selecting 25 tunnels at random (excluding tunnels at the edges of the arena) from the 22 day gallery image and measuring their width at the midpoint.
- $d$A tunnel segment was defined as the uninterrupted portion of a tunnel between nodes within a gallery system, with nodes being either tunnel intersection points, tunnel end points, the initiation site, or foraging sites.
- $e$Includes workers and soldiers.
Figure 3.1 Regression analysis of average wet weight against average head capsule width of termite workers from the four colonies used in the study.
Figure 3.2 Regression analyses of average worker wet weight against A) the number of tunnel segments created and B) the average width of tunnels created by colonies II, III, and IV.
Figure 3.3 Average length tunneled by colonies II, III, and IV on each day of the foraging trials. * Days on which tunneling means varied significantly ($P > 0.05$, PROC GLM, [SAS Institute 1999]). Means followed by the same letter are not significantly different (Tukey-Kramer HSD test).
While there were no differences in overall length, I did see significant differences during the early stages of gallery formation. On day one, colony II tunneled significantly more than colonies III and IV \( (F = 13.01; \text{df} = 2, 11; P = 0.0013) \), and on day two colonies II and III tunneled significantly longer than colony IV \( (F = 4.16; \text{df} = 2, 11; P = 0.0450) \). On day 12 colony IV tunneled significantly more than colonies II and III \( (F = 6.52; \text{df} = 2, 11; P = 0.0136) \). The length of tunnels constructed during each of the first four days of the 22-day foraging trials is regressed against worker weight in Fig. 3.4. On day one there was a strong positive relationship between worker weight and tunnel length. The relationship persisted through day two, but was beginning to weaken. By day three tunneling showed no correlation to worker weight. On day four the relationship shifted and there was a negative correlation, with smaller sized workers tunneling more than the larger workers. There was a significant relationship between tunnel length and the number of sites discovered on day one (Fig. 3.5).

3.4 Discussion

Excluding the weak termite colony involved in the study, I found evidence supporting the hypothesis that average worker size of a large foraging group has an effect on the group’s tunneling efficiency, and, in at least two aspects (tunnel width and tunnel segmentation), has an effect on tunnel pattern. My findings partly sustain research by Cornelius and Osbrink (2001) which demonstrated that after 21 days of tunneling, colonies of *C. formosanus* with greater average body weights created a larger tunneling area than colonies with lower average body weights. The size advantage in tunneling I
Figure 3.4 Regression analysis of average worker wet weight against length tunneled by colonies II, III, and IV on each of the first four days of foraging trials.
Figure 3.5 Regression analysis of length tunneled on the first day of foraging trials against the number of foraging sites discovered on the first day by colonies II, III, and IV.
observed was manifest during the initial period (first two days) of tunnel formation, and was not evident when comparing the final amount of tunneling. In fact, most of the tunneling by termites was done during the first week of the foraging trials. Initial rapid tunnel excavation tapering to limited tunneling activity has been reported by other researchers and was attributed to a lack of food stimulus within the foraging area (Hedlund and Henderson 1999). In the experiment mentioned above, foragers began with varying amounts of food and were allowed to tunnel outward into an area where no further food items existed. My foraging trials were designed such that termites began with only a small amount of food (filter paper) and were required to tunnel outward to discover additional food items arranged uniformly throughout the arena. We know from Figure 3.4 that healthy foraging groups consisting of larger individuals tended to tunnel more on day one. Additionally, Figure 3.5 shows that healthy foraging groups tunneling more on the first day tended to discover more food items. Integrating this information provides an explanation for the shift in slope of the regression lines over time in Figure 3.4. We can assume that as more food is discovered the stimulus to search for additional food resources decreases. Therefore, because the foraging groups with larger termites tunneled more in the beginning and discovered more wood earlier in the trials, their tunneling activity tended to peak sooner. Groups with smaller sized foragers found the same amount of food items by the end of the trials, but they were slower to discover the food items, and consequently their tunneling activities were more drawn out.

One proposed explanation for a size advantage in tunneling is that larger termites are better tunnelers because they have bigger mouthparts for transporting particles of substrate (Crosland and Traniello 1997). My measure of worker size, wet weight, did not
directly provide information relative to mouthparts, but the result of the regression analysis of wet weight on head capsule width indicated that termites with greater biomass did indeed have larger heads. Actual mouthparts were not measured, and we can only therefore presume that mouthpart size was positively associated with head capsule size. Large size probably provides some physical advantage in the mechanics of tunneling, but this benefit is more likely related to tunneling rate and not attributable to tunnel width or segmentation. Tunnel width is more a function of the number of termites focusing on a specific tunnel during its formation rather than the size of the mandibles relocating the particles of sand. Tunnel widths reported from this study are approximately one third of tunnel widths listed for *C. formosanus* by Puche and Su (2001a), who observed *C. formosanus* widening its tunnels after release into the foraging area. In general I did not observe workers widening existing tunnels. Additional areas of sand were excavated at the bases of some tunnels leaving the center initiation site, but because I measured segments at their midpoints, these areas did not affect my width measurements.

Alternatively, McMahan (1979) suggested that in comparison to smaller workers, larger termite workers possess larger sternal glands and produce higher pheromone titers which elicit stronger recruitment responses from other foraging individuals. This theory implies the following points: 1) Sternal gland size increases throughout the development of the worker, 2) Sternal gland size is directly related to pheromone concentration, and 3) Recruitment response is concentration dependent. While termite sternal glands have been shown to vary significantly in size between castes (Leuthold and Luscher 1974, Traniello and Busher 1985), there is little evidence of variation in gland size within a caste. Traniello and Busher (1985) reported a range of volumes for glands of the large worker
caste (third instar) of *Nasutitermes costalis* (Holmgren), but it is not clear whether the variation in gland volume was correlated to body size. Regardless, they did demonstrate a significant correlation between gland volume and recruitment activity within the third instar worker caste.

Considering this explanation, it is therefore conceivable that the differences in tunnel pattern and tunnel construction I observed between the three healthy colonies were partially due to variation in recruitment response to different concentrations of pheromone deposited by foragers. Of the tunnel features I measured, only tunnel segmentation and tunnel width were significantly correlated to average forager size. Given a set number of foragers, greater recruitment responses could lead to higher numbers of termites focusing at fewer sites of tunnel excavation. Such a scenario might produce a search tunnel pattern composed of fewer yet larger tunnels. Oppositely, with a weaker recruitment response, termites may forage in a less concerted manner, resulting in more sites of tunnel excavation, but with fewer termites operating at each location. In theory, the latter situation would produce a diffuse, more segmented search tunnel pattern with narrower tunnels. The differences in tunneling rate between the colonies during the first two days of the foraging trials could also be attributed to pheromone concentration. Grace et al. (1988) found trail following activity and rate of locomotion in *Reticulitermes hesperus* Banks to be dependent on the amount of pheromone present.

I can only speculate why colony I displayed significantly higher mortality. The manifestation of decreased vigor can be the result of a number of underlying factors, and it is not uncommon for variation in survival to occur between subterranean termite colonies during laboratory tests (Carter et al. 1972, Su and La Fage 1984b, Lenz 1985,
Lenz and Dai 1985, Thorne and Breisch 2001). It is interesting to note however that my finding that colonies composed of larger workers tunneled more effectively is counterintuitive to certain ideas on the developmental cycle of subterranean termite colonies. In theory, average worker size is smaller in young colonies due to a greater proportion of younger individuals in the foraging population resulting from high rates of reproduction. As the colony enters a declining phase, reproduction rates are lower, and the demographics begin to shift towards a population consisting of older individuals with greater body mass. Early work in Japan cited a connection between colony age and vitality, stating that young colonies composed of smaller workers are stronger than older colonies consisting of larger sized workers (Shimizu 1962). Additional support for this explanation is given by Grace et al. (1995). Tracking the average worker biomass and foraging population estimates of a single colony for 16 years, they demonstrated a strong relationship between the increase in average worker size and the decrease in the size of the foraging population. Results from the current study suggest that a colony’s foraging ability increases as its foraging population ages, however, there is an age threshold beyond which the foraging population begins to senesce and lose vigor. This would explain why colony I in my study displayed the highest mortality and tunneled the least compared to the other colonies even though it consisted of the largest workers. In a study using six different C. formosanus colonies, Cornelius and Osbrink (2001) also reported significantly higher mortality in the colony with the largest sized workers (5.39 ± 0.02 mg/worker).

Subterranean termite colonies exist in a complex environment that is subject to many factors that change over space and time. Factors such as environmental conditions,
interactions with predators and pathogens, availability of food resources, and inherited genetic traits may all singly or in combination influence foraging activities. The influence of these factors can be difficult to assess in the subterranean environment, but through experimentation in the laboratory it is possible to examine their affects individually. I found that the average size of workers within a colony of *C. formosanus* is at least one factor that affects tunnel width, tunnel segmentation, and tunneling rate. Unfortunately, due to the exclusion of one colony because of high mortality, this relationship was established using only three colonies. Tunneling comparisons between a greater number of colonies covering a broad range of average worker sizes would be ideal. Nonetheless, while the causes may not be entirely apparent, it is clear that differences in tunneling occur between Formosan subterranean termite colonies. Understanding the causes for these differences could aid in the control of subterranean termite infestations by tailoring strategies to the specific foraging behaviors of individual colonies.
CHAPTER 4

Tunnel Response by the Formosan Subterranean Termite (Isoptera: Rhinotermitidae) to Substrate Anomalies

4.1 Introduction

Subterranean termites operate in an underground environment rich with chemical, biological, and physical components. Some of these components interact favorably with termite foragers while others may hinder their progress as they tunnel through the soil in search of food. For example, work by Cornelius et al. (2002) showed that tunneling activity by *Coptotermes formosanus* Shiraki in sand was positively influenced by the presence of methanol extracts from fungus-infected sawdust. This supported much earlier work by Essenther et al. (1961) which suggested that wood, subjected to the natural processes of decay, could exude chemicals into the soil that are moved throughout the substrate by fluid transport, are attractive to termites, and are set-up as a gradient for termites to follow. On the other hand, research has suggested that certain plants may release chemical compounds which are repellent and, in some cases, toxic to termites. Sand containing dried vetiver root, *Vetivera zizanioides* Linn Nash, was shown to repel tunneling away from a food source. It was later determined that vetiver roots contained the compound Nookatone, which inhibited tunneling activity (Zhu et al. 2001).

In addition to chemical gradients, there is evidence that subterranean termites utilize physical objects during their exploration of the soil. Field observations had long suggested that subterranean termites use plant roots and cracks in the soil as tunneling guides (Greaves 1962, King and Spink 1969), but there was no empirical evidence until Singer-Pitts and Forschler (2000) showed that *Reticulitermes flavipes* (Kollar) and *R.*
virginicus (Banks) chose to tunnel along guide wires embedded in sand and in pre-made spaces significantly more than in areas of foraging arenas containing sand only. Evans (2003) similarly demonstrated that linear gaps in a two-dimensional substrate could significantly guide tunneling by Coptotermes frenchi Hill. Campora and Grace (2001) studied tunnel response to isolated objects in the substrate. Using foraging arenas containing C. formosanus, the authors found that in a homogeneous substrate tunnels radiated outward with no directional preference. However, once termites encountered a perimeter containing open vials, a significantly greater number of tunnels were concentrated in the areas where the vials were located. The resulting effect in arenas containing multiple vials was the systematic discovery of adjacent vials by repeated bursts of tunneling. The increased tunneling activity at the vials occurred regardless of whether or not the vials contained wood, suggesting that it was not the presence of a food resource that stimulated a tunneling response, but rather the physical presence of the vial itself.

The current study further investigates the properties of isolated objects in the soil that illicit tunneling responses when discovered by subterranean termites. Vials of different types were used in arenas to simulate the general characteristics of objects that may be encountered by termites as they tunnel through the soil, with the objective to determine which characteristics generate the greatest tunneling response.

4.2 Materials and Methods

4.2.1 Arena Design.

Tunneling behavior was studied in laboratory arenas with 31 by 31 by 0.25 cm
foraging spaces (Fig. 4.1). The foraging substrate consisted of silica sand [40-100 mesh (150-425 μm sieve), Fisher Scientific, Pittsburgh, PA] and distilled water (approximately 18% by weight). Six holes (3.1 cm in diameter) were arranged circularly around a larger hole (8.2 cm in diameter) in the center of the top layer of the arena. Plastic vials (capacity 48 ml) with the bottoms removed were placed in the six smaller holes and a similarly modified plastic jar (capacity 500 ml) was placed in the larger hole. Four arenas were used in the experiment, each containing a specific vial type (Fig. 4.2). Vial types were designed to create anomalies in the substrate that represented potential conditions termites may encounter in their natural tunneling environment. The following four vial types were used in the study: 1.) Open. This vial type simulated a pocket or empty space in the soil. Vials were empty and the substrate was removed down to the bottom layer of the arena directly beneath the vial. When termites encountered the perimeter of the vial they entered the inside space of the vial; 2.) Solid. This vial type simulated an unyielding non-food object in the soil. Vials extended down to the bottom layer of the arena. When termites encountered the perimeter of the vial they contacted the outside surface of the vial; 3.) Embedded wood. This vial type simulated a food object surrounded by soil. Vials were open but filled with the substrate and contained pre-weighed wafers (2.5 by 2.0 by 0.5 cm) of Douglas fir, *Pseudotsuga menziessii* (Mirb.) Franco. The embedded wafers rested on the arena’s bottom surface, and were positioned such that they faced the center of the arena. When termites encountered the perimeter of the vial they preceded forward in the sand until they contacted the wafer; 4.) Sand filled. This vial type represented a non-encounter. Vials were open and filled with substrate only. When termites reach the perimeter of the vial they come upon sand only.
Figure 4.1. Foraging arena design and set-up.
Figure 4.2 Vial types used in arenas to simulate substrate anomalies. A.) Open. Vials were empty and the substrate was removed down to the bottom layer of the arena directly beneath the vial. 2.) Solid. Vials extended down to the bottom layer of the arena. 3.) Embedded wood. Vials were open but filled with the substrate and contained pre-weighed wafers of Douglas fir. The embedded wafers rested on the arena's bottom surface, and were positioned such that they faced the center of the arena. 4.) Sand filled. Vials were open and filled with sand.
4.2.2 Protocol.

Termites used in the study were taken from three different colonies on the island of Oahu, Hawaii. Colony I was located on the windward side of the island at University of Hawaii Waimanalo Agricultural Experiment Station, and colonies II and III were found on the University of Hawaii Manoa campus, located respectively outside Miller Hall and the former publication building. Termites were extracted from their foraging territories, counted, and added to the experimental arenas within the same 24-hour period. One thousand five hundred termites (10% soldiers) were added to the center jar and allowed to tunnel outward towards the six smaller vials for a period of 48 hours. Four arenas, with one arena per vial type, were used in each separate trial of the experiment. Due to the small number of arenas, each trial was carried out with termites from one colony only. Colonies were alternated after each trial such that after the first three trials all three colonies had been used once. This cycle was repeated two times for a total of nine tunneling trials. During the tunneling trials, arenas were placed on a large light table in a dark room to illuminate tunnels and enable them to be photographed. Digital images (Coolpix 995 digital camera, Nikon, Melville, NY) of each arena were obtained at 24 and 48 hours after the addition of termites. After each trial, the arena contents were removed and the acrylic was cleaned with water and 90% ethanol. All tunneling trials were conducted in a facility where the ambient temperature fluctuated with the outside environment.

4.2.3 Analyses.

Tunnel surface area was calculated from digital images using ArcView 3.2 (ESRI Inc., Redlands, CA). Total tunnel area and the number of exit tunnels per vial were
compared for each vial type using the ANOVA procedure (SAS Institute 1999). Significant differences between colonies or trials were explored using the Tukey highly significant difference (HSD) test with an alpha level of 0.05. Circular statistics (Batschelet 1965) were used to examine the distribution of pooled exit tunnels from each vial type. Exit tunnels were pooled for each vial type, and the angle and magnitude were determined for the mean exit tunnel vectors. The Rayleigh test (Batschelet 1965) was used to determine randomness of exit tunnel distributions pooled for each vial type.

4.3 Results

Total number of tunnels exiting each vial and tunnel surface area were significantly greater in arenas with open vials than in arenas with solid, substrate filled, and substrate filled with wood vials ($F = 16.93; df = 5, 141; P < 0.0001$ and $F = 8.63; df = 5, 30; P < 0.0001$) (Figs. 4.3a and 4.3b). The solid vials had significantly more exit tunnels per vial than the substrate filled and substrate filled with wood vials, and there was no significant difference in number of exit tunnels between the substrate filled and substrate filled with wood vials (Figure 4.3a). The angle of the mean exit tunnel vector for all vial types ranged from $174^\circ$ to $198^\circ$ (Fig. 4.4). Exit tunnels were more concentrated around the mean exit angle in solid, substrate filled, and substrate filled with wood vials as evidenced by the value of exit tunnel vector, $r$ ($0.56, 0.49, \text{ and } 0.52$ respectively). The open vial type had a greater dispersion of exit tunnels as evidenced by a weaker exit tunnel vector of $0.30$. The Rayleigh test (Fig. 4.4) showed tunnel dispersion was non-randomly distributed around the mean exit tunnel angle from all vial types.
Figure 4.3 A comparison of mean (±SE) (A) exit tunnels per vial type and (B) total tunnel area in areas with different vial types. (bars with the same letters are not significantly different ($P = 0.05$; PROC ANOVA, Tukey’s HSD [SAS Institute 1999]).
**Figure 4.4** Circular statistics for pooled exit tunnels from each vial type. $\mu =$ angle of the mean vector, $r =$ magnitude of the mean vector, $s =$ standard deviation of the mean vector, and $p =$ the probability that exit tunnels are not randomly distributed ($P < 0.05$; Rayleigh Test [Batschelet 1965]).
4.4 Discussion

The results of this experiment demonstrate that pockets of space in the soil elicit tunnel creation by subterranean termites significantly more than solid objects or embedded food resources. Other research has identified subterranean termites' affinity for existing space in the tunneling environment (Pitts-Singer and Forschler 2000, Evans 2003), however this study is the first to suggest that space in the soil can lead to increased tunnel formation.

There are a number of possible explanations why open spaces in the substrate affected tunneling behavior. One reason may be that in the closed, artificial environment created by the foraging arenas, the open vials provided locations for the placement of excavated sand that were nearer to the sites of tunneling activity. In moist sand termites have been described to tunnel by pushing larger particles of sand to the side with their bodies and using their mouthparts to place the smaller particles along the tunnel wall (Ebeling and Pence 1957). On the other hand, in more compacted sand of smaller particle size, they must transport particles of the substrate from the leading edge of the tunnel back to a location where it will not impede traffic in the gallery system (Tucker et al. 2004). Voids in the substrate encountered during tunnel creation can serve as readily accessible soil deposition sites. Instead of having to travel all the way back to the center vial to deposit sand, foragers had the option to place sand particles in the intermediate spaces provided by the open vials. In support of this explanation, I observed that workers filled all discovered open vials with sand as tunneling progressed. It is possible that
using intermediate spaces for soil deposition can result in less travel time during the tunneling process, hence a faster tunneling rate.

It is also possible that the significantly greater amount of tunnel area in arenas with open vials was due to the fact that there were significantly more exit tunnels from open vials compared to other vial types. A void in the substrate creates an opportunity for foragers to initiate new tunnels in any direction. In a homogenous substrate, search tunnel networks are comprised of primary tunnels, with secondary and tertiary tunnels branching off at regular angles. Tucker et al. (2004) found that in less compacted soil, termites tended to initiate more secondary tunnels. The open vials in my experimental arenas created nodes in the tunnel network from which foragers could tunnel outward, essentially creating secondary tunnels, without having to branch off existing primary tunnels. In contrast, when termites discovered the solid vials, in order to continue in their original direction, they were constrained to tunnel along the surface of the object until they reached the other side. I observed that termites tended to tunnel the entire circumference of the vial upon contact before continuing onward. The ensuing ring around the solid vials may be one reason why there were significantly more exit tunnels from this vial type in comparison to the sand filled and embedded wood vials. Once termites had circumnavigated the vial, they could then go back and branch a new tunnel off at any point along the 360° path, a situation similar to that created by the open vial.

When termites discovered the embedded wood, they also encountered a solid object, but they created significantly fewer tunnels outward from the wood compared to the solid vials. Further, there was no significant difference between the average number of tunnels leaving the embedded wood and that of the tunnels leaving the sand filled vials
that were intended to represent a non-encounter. This can be partly explained by small amounts of tunneling that were observed vertically along the surface of the wood wafers. Due to the design of the arenas, these tunnels could not be measured, and therefore were not included in the calculation of the total tunnel areas. Another reason for the lack of exit tunnels from the vials with embedded wood may have been that as the wafers were discovered, foragers began to allocate a certain amount of energy to feeding and less energy to tunneling. Tunneling by subterranean termites has been shown to be inversely related to the amount of food resources available (Hedlund and Henderson 1999) and C. formosanus in particular maintains a strong preference to remain with discovered food (Delaplane and LaPAGE 1989, Cornelius and Osbrink 2001).

Pooling all exit tunnels from each vial type demonstrated that tunnels leaving a discovered object generally maintained the same direction as the tunnel or tunnels that first encountered the object. This directional effect was more predominant when a solid non-food object was encountered, and weakest when an open space was encountered. The absence of a significant directional effect is defined as random (Batschelet 1965), however, the results of the Raleigh test reveled that this was not the case for tunnels leaving the open vials. Even though the open vials elicited tunnels in many directions, the overall distribution of exit tunnels was not random because workers did not initiate tunnels immediately backwards.

If given the choice to enter a preexisting space or create their own space, subterranean termites will generally choose the former option. When the space is linear such as a pre-made tunnel by another subterranean organism, a gap along the surface of a linear object, or a crack in the substrate, termites will follow it to its end and continue
tunneling onward in the same direction (Evans 2003). If the space is a discrete pocket, then foragers will commence to dig multiple tunnels fanning outward away from the point of entry. Components of the underground environment such as voids and to some extent solid non-food objects promote the creation of additional search tunnels. This effect was not seen with embedded food objects, although it is possible that as a food resource is consumed over time, termites will begin to forage outward in search of new resources. These findings give further support for discouraging the practice of backfilling with debris during construction, even if the debris is inorganic, because of the resulting free space in the soil that it creates.
CHAPTER 5
Comparison of Tunneling in the Laboratory and Field by Foragers of the Formosan Subterranean Termite

5.1 Introduction

Various aspects of tunneling by subterranean termites (Isoptera: Rhinotermitidae) have been studied in the laboratory using two-dimensional arenas and foragers from laboratory colonies (Reinhard et al. 1997, Robson et al. 1995, Pitts-Singer and Forschler 2000, Hedlund and Henderson 1999) or discrete groups of foragers extracted from field colonies (Su and Puche 2003, Puche and Su 2001a, Puche and Su 2001b, Puche and Su 2001c, Tucker et al. 2004, Cornelius and Osbrink 2001), but there is little information on tunneling by foragers that are a functional part of a large field colony (Evans 2003). Many of the authors of the works referenced above recommend caution in interpreting their results due to the discrepancies of scale and the number of foragers used. Utilizing a small number of foragers in a small space can lead to a considerable criticism of conclusions (Evans 2003). This presents a problem in the area of termite control since the increased popularity of non-repellent treatments has made our understanding of subterranean termite foraging behavior an important aspect of termite management, and the use of artificial arenas is one of the most practical means to investigate termite tunneling behavior as they search for food.

Puche and Su (2001b) approached this dilemma with fractal analysis and found that fractal geometry could be an effective method to analyze tunneling systems independent of scale. Using this method with artificial arenas they found that tunnel abundance, or the area of space explored, did not significantly increase past a certain
threshold number of foragers. This was determined to be approximately 1,000 termites/arena. In addition, they showed that tunnel complexity remained unchanged between foraging groups ranging from 250 termites to 2000 termites (Puche and Su 2001c). Evans (2003) addressed the issue by using foragers from an actual field colony of *Coptotermes frenchi* Hill; however, the colony used was confined to a large steel drum and therefore was not truly representative of a typical field colony actively searching for food within its underground foraging territory.

My objective in this study was to investigate the differences that exist in tunneling between small groups of foragers in the laboratory and foragers in the field environment. I set up two-dimensional foraging arenas in the laboratory and in the field directly over active foraging sites of two colonies of the Formosan subterranean termite, *C. formosanus*. Observations were made on daily tunneling, and the amount of wood consumed was analyzed. In addition, I closely monitored tunneling in the field over a three-day period to determine whether tunneling activity followed a circadian rhythm.

5.2 Materials and Methods

5.2.1 Arena Design.

All arenas used in the laboratory (Fig. 5.1b) were identical to those used by Campora and Grace (2001) in previous tunneling research, with foraging sites containing pre-weighed wafers of Douglas fir, *Psuedotsuga menziessii* (Mirb.) Franco. Arenas
Figure 5.1 Arena design and set-up in the field (A) and laboratory (B).
implemented in the field were constructed using the same materials as the laboratory arenas, but were slightly modified in design (Fig 5.1a). The field design was altered such that when installed over an active Formosan foraging site, termites entered the arena from a hole in the bottom rather than the top. To seal the bottom, a corrugated cardboard disc was affixed directly over the entry hole with an adhesive. To minimize variation between the two arena types, identical cardboard discs were added in the same manner directly under the entry hole in laboratory arenas. The active foraging sites in the field were previously maintained with Douglas fir box traps, using a modified version of the protocol described by Tamashiro et al. (1973). Before installation of the arenas, the box traps were removed and a new Douglas fir stake was driven in approximately to ground level. A much smaller box constructed of Douglas fir (10.8 by 8.6 cm) was then placed over the stake. Corrugated cardboard, tightly rolled to the same diameter as the arena entry hole, was inserted into the box so that the bottom was flush with the bottom of the box and the top extended out 9.5 cm. The top section of the cardboard roll was covered by PVC pipe so that only 7.0 mm of the tip protruded. A plastic bucket with the bottom removed was placed over the box/cardboard/PVC assemblage (Fig 1.A). The arena was then mounted such that it rested on the rim of the bucket and PVC pipe, with the protruding tip of the cardboard roll inserted into the arena entry hole such that its surface was flat against the cardboard disc. Four additional buckets were placed at the corners of the arena to provide support. Once installed the entire structure was housed in a plywood box (1.25 by 1.25 m by 0.40 m).

5.2.2 Protocol.
Three arenas were installed over active foraging sites in each of two territories occupied by separate *C. formosanus* colonies, colony A and colony B. Colony A was located at the University of Hawaii agricultural experiment station in Waimanalo (Oahu), and colony B was located on the University of Hawaii campus at Manoa. Three separate arenas for each colony were also set up in the laboratory with groups of 1,500 termites (10% soldiers) added to each. Termites for laboratory use were extracted, counted, and added to arenas on the same day. Termites in both the field and laboratory arenas were allowed to tunnel for a period of 7 days. In contrast to the laboratory arenas, which were permanently backlit, field arenas were backlit with fluorescent lighting only during the periods when photographs were taken. Digital photographs of tunnel galleries were taken each night (Coolpix 995 digital camera, Nikon, Melville, NY). During one 72 h period, tunneling in two field arenas was monitored at three-hour intervals within one colony to examine temporal fluctuations in tunneling rate. During daylight hours of activity, the plywood arena housings were first covered with 5 mm black plastic sheeting to maintain a dark environment. In the absence of sunlight, the housing lids were removed and the arenas were photographed.

Air temperature immediately outside laboratory and field arenas was recorded during all tunneling trials with HOBO H8 Pro Series data loggers (Onset Computer Corporation, Bourne, MA). Laboratory arenas were not in a temperature-controlled facility and were subject to heat fluctuations related to the outdoor environment. Tunneling trials were conducted on different dates over a period from June 8 to October 30, 2001 (colony A field: June 8 - July 17; colony A lab: August 29 - September 9; colony B field: September 15 - October 5; and colony B lab: October 23 - October 30).
At the end of every field and laboratory trial, wafers were removed from the foraging sites of each arena, cleaned, and weighed to determine wood loss. Population estimates were obtained upon completion of all tunneling trials for both colonies using a triple mark-release-recapture method (Begon 1979).

5.2.3 Analysis.

Tunnel surface area was calculated from images using ArcView 3.2 GIS software (ESRI, Redlands, CA), and search patterns were compared between field and lab arenas. Analysis of variance using the MIXED procedure (SAS Institute 1999) was used to create a fixed effects model to determine the effects of colony and treatment (arena type) on total area tunneled and wood consumed.

5.3 Results

I did not quantify tunnel pattern or complexity, but there were no visually obvious differences in the general pattern of resource discovery between termites in the laboratory and the field. Tunneling in both field and laboratory arenas followed a similar pattern with tunnels radiating out from discovered feeding sites, then intercepting nearby sites and radiating out again in sequential fashion (Fig. 5.2). This pattern sequence was consistent with previous laboratory arena studies (Campora and Grace 2001), except tunnels entering the arena were not randomly distributed. This was due to the corrugated cardboard discs located at the termites’ entry point into the arenas. Tunnels entering arenas in both the laboratory and field portions of this study continued outward with the same orientation of the corrugations of the center cardboard disc. Although tunneling patterns were similar in both the field and laboratory, there was a significant colony by
treatment effect on total area tunneled \( F = 107.42; \text{df} = 1, 8; P < 0.0001 \) (Fig. 5.3) and on the amount of wood consumed \( F = 151.75; \text{df} = 1, 8; P < 0.0001 \) (Fig. 5.4).

Temperature varied among foraging trials with the largest difference between the two laboratory tests (Fig. 5.5). Tunneling rate in the two arenas was erratic over the 72 h period for which I collected data at 3 h intervals, but it appeared to be related to temperature with a lag effect (see Fig. 5.6). Population estimates for colonies A and B at the termination of experiments were \( 3,184,286 \pm 469,675 \) and \( 2,072,475 \pm 105,077 \), respectively.

### 5.4 Discussion

These results indicate that tunnel patterns of the Formosan subterranean termite are comparable whether observed in the field or laboratory. The only exception was that the initial direction of tunnels entering the arena was not random, and was oriented in the direction of the corrugations of the center cardboard disc. This supports the suggestion that subterranean termites not only utilize guides when tunneling (Pitts-Singer and Forschler 2000), but that they may continue tunneling in the general direction that guides are oriented even though the guide may have ended.

I also demonstrated that differences in rate of tunneling can exist between colonies in the field. There are a variety of factors that can influence a colony’s foraging behavior in the field. Two of the most import factors may be temperature and moisture (Haverty et al. 1974). Temperature is somewhat confounded in this study due to the fact that tunneling trials were conducted on different dates in settings where the temperature was uncontrolled and fluctuated with the outside environment. Temperatures recorded
Figure 5.2 Images of field and laboratory arenas with digitized tunnels showing the sequence of gallery formation representative of both colonies. Tunnels shown in blue are primary tunnels originating from the center. Secondary tunnels (green) originate from sites discovered by primary tunnels. Tertiary tunnels (yellow) originate from sites discovered by secondary tunnels. Quaternary tunnels (orange) originate from sites discovered by tertiary tunnels.
Figure 5.3 Mean cumulative tunneling by colonies in the field and laboratory.
Figure 5.4 Mean wood consumption by colonies in the field and laboratory. Means with the same letter are not significantly different.
Figure 5.5  Temperatures recorded during laboratory and field tunneling trials.
Figure 5.6 Comparison of tunneling activity and temperature of two field arenas over a three day period
during each of the trials, however, were not substantially different. In fact the greatest
difference in temperature occurred between the two laboratory trials, and in these trials
there were no significant differences in area tunneled.

Arenas in the field and laboratory were equally moist; therefore, if tunneling in
the arenas was affected by water, the effect must have been related to differences in water
needs between the colonies. Puche and Su (2001a) provided evidence that in the
laboratory, tunneling foragers are attracted to soil moisture gradients. If colony A was
located in an area without water readily available, this could explain its much stronger
tunneling response when entering the moist sand of the arenas. The two field locations in
this study were similar with regard to precipitation, but I can only speculate on the
amount of water that was actually available to the two colonies. Haagsma and Rust
(1995) suggested that when compared to undisturbed environments, urban environments
are more stable with respect to abiotic factors such as soil moisture. Under this
assumption, colony B, being situated in an urban setting, could have possibly been
provided access to predictable, artificial sources of moisture. This would give the
foragers from colony B no added incentive to tunnel in moist sand. It should be noted,
however, that even though colony A was in a less urban setting, it was not located in a
completely undisturbed environment. Because colony A was situated in an agricultural
area not far from irrigated fields, it was most likely not too dissimilar from colony B with
regard to water availability in the field.

Differences in foraging activity by subterranean termites related to temperature
and moisture are more typically correlated to seasonal differences (LaPage et al. 1973,
Haverty et al. 1974, Delaplane et al. 1991, Haagsma and Rust 1995). In Hawaii, these
factors do not fluctuate to the extent that they do in temperate areas. Furthermore, while some seasonality does occur in Hawaii, there were no major shifts in temperature or precipitation observed during the period of time over which all the trials were conducted. The fluctuations in tunneling activity I observed throughout the day in the two arenas of Colony A did appear to be directly related to temperature. The apparent time lag between increased tunneling and ambient air temperature may be due to the fact that foragers are responding to the soil temperature just below the air/soil interface.

It is more likely that factors responsible for the differences I observed between the two colonies in the field were related to conditions intrinsic to each colony (i.e. population size) and the biotic environment (i.e. available food resources) rather than environmental and abiotic conditions. Assuming the populations estimates provided correct approximations of the colonies' relative sizes, colony A's greater tunneling activity and wood consumption in the field were possibly reflective of a greater need for food to sustain its larger foraging population. Another explanation could lie in the location of the arenas within the two colonies' foraging territories. Research with *Macrotermes bellicosus* (Smeathman) has also shown that foraging pressure is not necessarily equally distributed throughout a colony's foraging territory (Traniello and Leuthold 2000). This termite spatially allocates foraging and concentrates on certain sectors of its gallery system. Similarly, feeding activity by *C. formosanus* has been shown to vary throughout feeding sites within a colony’s foraging range (Grace et al. 1996). In the current study, the location of the arenas within the foraging territories could have determined how many foragers entered them. Perhaps by chance I placed arenas
over a hot spot of colony A’s territory where many workers were being allocated at that
time, and I may have placed the arenas over an area of low activity in colony B’s range.

There is also evidence to suggest that tunneling by a group of Formosan
subterranean termites is negatively correlated to the amount food available (Hedlund and
Henderson 1999), therefore if one colony had more food resources to utilize than another,
it might not be as quick to explore new areas for food. Due to the cryptic nature of
subterranean termites, it is not possible in the field to determine where all of their feeding
sites are, but it is possible that a primarily urban environment may provide singular food
resources on a large scale, such as man-made wooden structures, that do not promote
searching behavior to the same extent as a natural/agricultural environment which
provides smaller scattered food items.

My results also demonstrated that the threshold of 1,000 workers/arena
established by Puche and Su (2001c) does not necessarily apply to arenas used in the
field. Fractal analysis was not applied to the tunnel networks, but in terms of total tunnel
area, the amount of tunneling was significantly greater in field arenas with an indefinite
number of foragers compared to laboratory arenas containing 1,500 termites per arena.

In conclusion, I found that the basic spatial pattern of tunneling by C. formosanus
foragers is comparable when observed in the field or laboratory. Furthermore, tunneling
activity of C. formosanus foragers fluctuates throughout the day and is possibly related to
temperature. Differences in daily tunneling rate can occur between colonies of C.
formosanus in the field that are not apparent when making comparisons using equal
numbers of foragers in the laboratory. In this study the factors affecting tunneling in the
field were probably those that were intrinsic to the colony such as forager population
size, age of the colony, resource availability, and the spatial allocation of foragers to available food resources. This variation of tunneling activity between colonies in the field provides evidence that some colonies may be more difficult to control than others when using non-repellent termiticides, particularly in ground baits.
CHAPTER 6

Behavioral response of the Formosan subterranean termite to borate treated wood

6.1 Introduction

It is well documented that subterranean termites exhibit a concentration-dependent avoidance behavior to certain non-repellent and slow acting toxicants. Various authors have demonstrated termite avoidance thresholds based on the concentration of termiticides such as mirex (Su and Scheffrahn 1991), sulfuramid (Su and Scheffrahn 1991), hydramethylnon (Su et al. 1982), a dihaloalkyl arylsulfone biocide (Su and Scheffrahn 1988a), and boron in the form of boric acid and boron salts (Grace 1990, Grace et al. 1990, Jones 1991). However, it is not well known exactly why termites eventually shun these compounds after initially showing no signs of avoidance. This leads to one of the more interesting questions emerging from the continued development of non-repellent termiticides. When delayed avoidance occurs, are foragers simply reacting to a direct stimulus, or are they learning to recognize and avoid slow acting toxicants from experience?

One explanation in support of a direct stimulus response is the tendency of termites to avoid areas where dead foragers are present. Observing foragers tunneling through agar filled petri dishes, Su et al. (1982) discovered that if mortality from exposure to a termiticide occurred at a rate fast enough such that termites died and remained at the site of exposure, then subsequent foragers would avoid that site because of the presence of dead foragers. Thus, even though the termiticide itself provided no stimulus for avoidance, termites shunned the treated area due to the repellency of the
termite corpses. This behavior was termed “necrophobia” and was found to occur with non-repellent termiticides possessing a fast-acting mode of action such as chlorpyrifos and chlordane (Su 1982). This phenomenon was also described to occur in baiting situations with the slower acting non-repellent toxicants abermectin and zinc borate. Laboratory assays with the latter chemicals revealed that termite mortality occurred locally around the bait sites, causing termite activity in the vicinity of the sites to cease for period of time (Forschler 1996).

A second possibility for avoidance of non repellent, slow acting termiticides is that termites learn to avoid treated areas as a result of sub lethal exposure. In theory, individuals that are exposed to a non-repellent termiticide, but do not completely succumb to the chemical’s deleterious effects, are able to formulate a relationship between the negative effects they experienced and the chemical treatment. Su et al. (1995a) attributed this behavior to “associative learning” and defined it as “the ability to form associations between previously meaningless stimuli and reinforcements”. Similarly, Thorne and Breisch (2001) termed the behavior “aversion” and explained it as a “learned response after an experiential association between one or more of the compound’s attributes and a negative impact such as sickness”. The occurrence of associative learning or aversion has been suggested for the avoidance of feeding on such non-repellent treatments as sulfuramid treated boards (Su and Scheffrahn 1991) and hydramethylnon baits (Su et al. 1982).

Borate in the form of disodium octaborate tetrahydrate (DOT) is similarly categorized as a non-repellent, slow acting toxicant. Tunneling assays using DOT powder (TIM-BOR) have demonstrated that when mixed with sand, DOT did not inhibit
tunneling by subterranean termites, but it did cause the eventual mortality of a substantial number of foragers (Grace 1991). This form of borate is more commonly used in the pretreatment of lumber and wood products intended for use in interior construction (Grace 1997). When pressure treating wood with a preservative, deterrency of feeding is the desired effect; therefore, the intent is to treat with concentrations of DOT that prevent termite feeding. Even when treated with relatively high DOT concentrations, however, a small amount of feeding by termites still occurs. Grace et al. (1992) found that wood treated at the concentration of 1.02 % Boric Acid Equivalents (BAE) and exposed for 23 weeks to a field colony of the Formosan subterranean termite, Coptotermes formosanus Shiraki, remained structurally sound, experiencing only 2.5 % weight loss, but some cosmetic damage did occur. In a further effort to determine the cumulative effect of this tasting behavior, Grace and Yamamoto (1994) sequentially exposed wood treated at various high concentrations of DOT to four C. formosanus colonies for 10 weeks each. Similar to the previous study, wood treated at the highest concentration of 2.52 % BAE experienced less than 1 % wood loss after the entire 40 weeks, but there was evidence of tasting by all four colonies at the end of each 10-week exposure. It was hypothesized from these results that the higher concentrations of DOT were causing termites to die in the immediate vicinity of the treated wood, creating avoidance by the remaining foragers.

The purpose of the study reported here was to observe the behavior of the Formosan subterranean termites as they discovered wood treated with DOT and determine if avoidance to wood treated at label rates (HI-BOR) developed because of necrophobia or associative learning as result of sublethal exposure. To accomplish these objectives observations of termite foragers were made both in field and laboratory
settings using two-dimensional foraging arenas containing treated and untreated wood. This provided a means to establish where foragers were dying within the gallery system and to examine the corresponding distribution of the surviving foragers relative to the treated wood. Additionally, I examined the spatial importance of treated wood within a gallery system and explore the possibility that learning may occur in response to the location of a toxicant and not from recognition of the toxicant itself.

6.2 Materials and Methods

6.2.1 Arena Design and Set-up.

Arenas used in the field (Fig. 5.1a) were slightly modified from the design utilized in the laboratory. The field design was altered such that when installed over an active Formosan foraging site, the termites entered the arena from a hole in the bottom rather than the top (Fig. 5.1b). A corrugated cardboard disc was affixed over the entry hole with an adhesive (Household Goop. Eclectic Products Inc, Pineville, LA) to keep the substrate inside the arena from falling out. Identical cardboard discs were also added to the center of the laboratory arenas to minimize variation between the two arena types. Aside from the cardboard disc, arenas used in the laboratory (Fig 5.1b) were identical to those described by Campora and Grace (2001) in previous research on tunneling behavior. Silica sand [40-100 mesh (150-425 μm sieve), Fisher Scientific, Pittsburgh, PA] and distilled water (approximately 18% by weight) were added to both laboratory and field arenas to serve as the foraging substrate.

Wood added to the arenas was supplied by U.S. Borax and consisted of wafers of Douglas fir, *Pseudotsuga menziessii* (Mirb.) Franco, cut to AWPA (2003) standards (25 by 25 by 6 mm). Borate treated wafers were created by the addition of a 17.0 % Tim-bor
solution and subjected to a 15 minute vacuum and half hour press cycle. Chemical assays were performed on a random sample of 12 treated wafers and produced a mean of 1.76 % (± 0.16 %) boric acid equivalents (BAE).

Termites used in the laboratory foraging trials were obtained from a colony located on the University of Hawaii Manoa campus near Miller Hall. Laboratory arenas were maintained in a dark room where the temperature fluctuated with the outside environment. Field foraging trials were conducted over a colony located on the windward side of Oahu at the University of Hawaii Waimanalo agricultural experiment station.

6.2.2 Protocol.

Six arenas were set up in the laboratory and three arenas were set up in the field. Three of the laboratory arenas initially contained borate treated wafers on the west side, and the remaining three arenas served as controls and contained no treated wafers. All three of the arenas used in the field initially contained treated wafers on the west side. Termites were allowed to forage in all arenas for a period of three weeks. Wood wafers were replaced in the arenas as shown in Figure 6.1. After one week in the field and two weeks in the laboratory, the treated sides of the arenas were switched from the west side to the east side. Wood wafers were replaced for a third time in the field arenas such that the west side again contained treated wood during the final week.

6.2.3 Analysis.

The variables measured in the laboratory were tunnel distribution, forager distribution, mortality distribution, forager presence on wood, and wood consumption.
Figure 6.1 Location and replacement protocol of treated and non treated wood wafers in laboratory treatment (A), laboratory control (B), and field (C) arenas.
Tunnel distribution was determined from digital images of the arenas taken at 24 hour intervals. Using ArcView 3.2 GIS software (ESRI, Redlands, California), daily tunnel formation was digitized and quantified by the amount of tunnel surface area in the east and west sides of the arenas. Forager distribution in the arenas was measured from counts of termites made within a 5 by 5 cm grid. Counts were made daily between 3:00 and 5:00 pm. Termites counted consisted of all workers and soldiers within each grid square. To facilitate estimates, termite numbers within each grid square were classified into 6 categories (0 = 0, 1 = 1, 2 = 2 to 12, 3 = 13 to 24, 4 = 25 to 50, and 5 = 51 or more). Because the counts represented snapshots in time of termites as they moved throughout the gallery system, contour maps were extrapolated from the count data in ArcView using the Inverse Distance Weighted method. The volume of the resulting termite density landscape was then calculated for both sides of the arenas. The 5 by 5 cm grid was also used to determine mortality on each side of the arenas. Counts of dead termites were made daily in each grid square, and the totals for each side were compared. Forager presence on wood was measured daily by examining each wafer for the presence of termites. Data were recorded as percent coverage of the wafer by termites to the nearest 10 percent. The coverage on all wafers was pooled and divided by the total possible coverage to obtain the overall percent coverage of wood on each side. All wafers used in the experiment were pre-weighed; therefore, wood consumption was calculated by weighing the wafers after exposure to termites in the arenas.

Only tunnel distribution, mortality distribution, and wood consumption were measured in the field. Methods used to measure these variables were identical to those employed in the laboratory arenas. Analysis of variance (PROC GLM, SAS Institute
1999) was used to determine significant differences in tunnel distribution and wood consumption on each side of the arenas. Means were compared using the Tukeys Highly Significant Difference (HSD) test. The other variables measured in the laboratory arenas were compared separately for each trial due to the fact that behaviors varied in each arena depending upon the timing of discovery and amount of feeding on treated wafers.

6.3 Results

6.3.1 General Behavior.

Termites in laboratory arenas were generally engaged in one of the four following activities: 1) actively feeding on wood; 2) traveling back and forth within the gallery system, actively excavating sand during tunnel formation and depositing it elsewhere within the arena; 3) traveling singly throughout the gallery system with no apparent goal or destination; and 4) resting in large groups at specific locations within the gallery system. During the first days, most of the termites visible in the arenas were involved in tunneling, with the remainder of the group remaining inside the entry jar. Tunneling activity tapered off after the first week, and most of the termites were found either in resting groups or feeding on the wafers. Resting groups tended to be around the perimeter of the entry jar, but sometimes occurred elsewhere within some of the larger tunnels. Tunneling activity after the first week was sporadic and occurred in localized areas conducted by small groups of workers.

While the behaviors observed above generally occurred in the same manner for each arena, the sequences of events were not identical, especially for the arenas that
Figure 6.2 Time of discovery for each wafer in all laboratory tunneling trials. The number inside each circle represents the day of discovery, and the number outside each circle at the bottom right represents the wafer location.
contained treated wood. To allow for better interpretation, Figure 6.2 provides information regarding when wafers within laboratory arenas were discovered, and the results from each laboratory tunneling trial are reported separately in Figures 6.3 through 6.8.

6.3.2 Individual Laboratory Arenas

6.3.2.1 Treated Laboratory Arena 1

*Days 1 through 14.* A total of 11 wafers were discovered. Foragers found 7 wafers on the first day (three treated wafers on the west side and four untreated wafers on the east side), and two more treated wafers and one additional untreated wafer on the second day (Fig. 6.2). No more wafers were discovered until day 7, when the last untreated wafer was encountered (Fig. 6.2). Termites were distributed equally on both sides of the arena until day four, when more were present on the east side (Fig. 6.3). By day 7, almost all of the termites were on the east side, and this distribution pattern persisted until day 14 (Fig. 6.3). Data on wood coverage in Figure 6.3 suggests that termites fed on some of the treated wafers on the first day, but most feeding occurred on the untreated wafers. From day four to day 14 there was no feeding on treated wafers. Tunnels were distributed equally on both sides of the arena up until day 7 after which all new tunneling was on the east side (Fig. 6.3). Signs of mortality appeared on day 5, and were most prevalent on the east side of the arena. The number of dead termites on the east side increased steadily through day 11 and then leveled off at about 300 individuals through day 14 (Fig. 6.3). The number of dead termites on the west side reached a peak of 50 on day 8 and remained unchanged through day 14 (Fig. 6.3).
Figure 6.3  Termite distribution, feeding, tunneling area, and mortality on each side of the first laboratory treated arena.
Days 15 through 21. After all wafers were replaced such that treated wafers were now on the east side, termite distribution returned to being equal on both sides by day 16 (Fig. 6.3). The day after new wafers were placed in the arena there were no termites present on any of the wafers; however, by day 16 termites began returning to the east side that contained treated wafers. No termites returned to wafers on the west side (Fig. 6.3), and termites did very little tunneling after day 14. A sharp increase in mortality was observed from day 16 through day 21 (Fig. 6.3).

6.3.2.2 Treated Laboratory Arena 2

Days 1 through 14. A total of 7 wafers were discovered (Fig. 6.2). All seven were discovered in the first two days and included four treated wafers on the west side and three untreated wafers on the east side (Fig. 6.2). The number of termites on treated and untreated wafers on the first day was similar to Arena 1 with about 10 % of treated wood covered and 30 % of untreated wood covered, but feeding on treated wood began to intensify on day two, with 35 % coverage (Fig. 6.4). By day three a decreasing trend in numbers occurred and on day five there were no termites on treated wood (Fig. 6.4). Termites remained on untreated wood until day eight, when their presence on wood on the east side started to decline (Fig. 6.4). Also at this time they again began to be present on treated wood on the west side (Fig. 6.4). By day 14, there was an observed 15 % coverage on the west side, and no termites were present on wood on the east side (Fig. 6.4). Tunneling was approximately equal on both sides until day 8, at which time tunneling activity was seen only on the east side. There was no further tunneling activity after day 9, and signs of mortality in arena two were first seen on day four (Fig. 6.4).
Figure 6.4 Termite distribution, feeding, tunneling area, and mortality on each side of the second laboratory treated arena.
Numbers of dead termites increased on both sides of the arena through day 12, but the increase was greatest on the east side of the arena (Fig. 6.4). Mortality within the arena was stable on both sides by day 14 (Fig. 6.4).

Days 15 through 21. Most of the termites in arena two were dead by day 15, and by day 18 all termites had died; consequently, after the replacement of wood in arena 2 there was very little observed activity (Fig. 6.4).

6.3.2.3 Treated Laboratory Arena 3

Days 1 through 14. A total of 11 wafers were discovered, 6 wafers on the west side and 5 on the east side (Fig. 6.2). On the first day, all 5 wafers on the east side were discovered, and only one wafer on the west side was discovered (Fig. 6.2). The next 5 wafers on the west side were found on days 5, 7, 8, and 10 (Fig. 6.2). Termites were distributed more on the east side of the arena during the first four days of the trial, but by day 5 the distribution was approximately equal on both sides with some fluctuation through day 14 (Fig. 6.5). With the exception of a small number of individuals on one of the treated wafers on day 5, termites were almost exclusively feeding on untreated wafers until day 11 (Fig. 6.5). At this time they began feeding on a treated wafer discovered on the preceding day. From day 11 to day 14, termites were also seen feeding on treated wafers located at sites one and 6 (Fig 6.5). During the first four days, tunneling activity was focused on the east side (Fig. 6.5). After day four tunneling was focused on the west side, tapering off through day 11 (Fig. 6.5). The first dead termites were seen on the west side of the arena on day 9 (Fig. 6.5). Mortality slowly increased on both sides of the arena until day 13, when termites started dying at a faster rate on the east side (Fig. 6.5).
Figure 6.5 Termite distribution, feeding, tunneling area, and mortality on each side of the third laboratory treated arena.
Days 15 through 21. Termites were found on both sides of the arena through day 21; however, during this time they only fed on newly added treated wafers located on the east side (Fig. 6.5). Very little tunneling was observed after day 14 (Fig 6.5), but one additional wafer was discovered on the west side on day 17 (Fig. 6.2). Mortality continued to increase sharply, with more termites dying on the east side, up through day 21 (Fig. 6.5).

6.3.2.4 Control Laboratory Arena 4

Days 1 through 14. A total of 10 wafers were discovered. Four wafers were discovered on each side during the first day of tunneling (Fig. 6.6). Two additional wafers were discovered on the east side on the second and ninth days. This was correlated with a higher amount of area tunneled on the east side during this time (Fig. 6.6). Tunneling on the west side did not increase substantially after day 6 (Fig. 6.6). In general, termites displayed no obvious preference for either side of the arena, and foraging was approximately equal throughout.

Days 15 through 21. Little difference was observed compared to the first two weeks, except for a drop in wood coverage on both sides of the arena during the first two days after wood was replaced (Fig. 6.6). An increase in tunneling activity occurred between days 14 and 15 on the west side (Fig. 6.6), resulting in one additional wafer on the west side being discovered (Fig. 6.2).
Figure 6.6 Termite distribution, feeding, tunneling area, and mortality on each side of the first laboratory control arena.
Figure 6.7 Termite distribution, feeding, tunneling area, and mortality on each side of the second laboratory control arena.
6.3.2.5 Control Laboratory Arena 5

Days 1 through 14. Tunneling on the first two days occurred almost entirely on the west side, resulting in the discovery of 5 wafers on the west side and only two wafers discovered on the east side (Fig. 6.2). By the third day, termites increased tunneling efforts on the east side (Fig. 6.7). This trend continued through day 11 (Fig. 6.7), resulting in the subsequent discoveries of wafers on the east side on days four, 5, and 14 (Fig. 6.2). Additional wafers on the west side were discovered on days three and 12 (Fig. 6.2). Wood coverage reflected the distribution of tunneling with termites feeding on west side wafers almost exclusively for the first three days, and then beginning to move over to east side wafers as they were discovered over time (Fig. 6.7). Even though it fluctuated, percent coverage on wood discovered on the west side was always greater than coverage of wood on the east side (Fig. 6.7). Termite distribution was heavily skewed to the west side during the entire first two-week period (Fig. 6.7)

Days 15 through 21. Very little tunneling occurred during this period, and the majority of termites within the arena remained on the west side (Fig. 6.7). The first day after the wood was replaced, no termites were present on the newly added wafers (Fig. 6.7). Feeding resumed on day 16 and continued through day 21, however, foragers did not return to wood on the east side as frequently as they did on the west (Fig. 6.7).

6.3.2.6 Control Laboratory Arena 6.

Days 1 through 14. Tunneling was initially greater on the east side (Fig. 6.8), and on the first day four wafers were discovered on the east side compared to three wafers found on the west side (Fig. 6.2). By day 6, the area tunneled on both sides was
Figure 6.8 Termite distribution, feeding, tunneling area, and mortality on each side of the second laboratory control arena.
approximately equal (Fig. 6.8), with two more wafers being discovered on the west side and one more wafer discovered on the east side (Fig. 6.2). An additional wafer was discovered on the east side on day 9 (Fig. 6.2). Very little tunneling occurred after day 10 (Fig. 6.8). Termites showed considerable preference for wafers on the west side during the first 8 days, but by day 9 feeding was equal on both sides (Fig. 6.8). Total distribution of termites was slightly higher on the east side during the first 5 days, but then shifted on days 8 to 14 to pattern with more termites located on the west side (Fig. 6.8).

*Days 15 through 21.* Termites again did not return to the newly added wafers on the first day after wood replacement, but they slowly resumed feeding as coverage on wood increased equally on both sides through the end of the trial (Fig. 6.8). There was no additional tunneling activity during this period, and termites were approximately equally distributed on both sides (Fig. 6.8).

### 6.3.3 Field Arenas

Tunneling activity in all field arenas began to taper off at day 6, one day before the first wood replacement and switch of treated wafer locations (Fig 6.9). Tunnel monitoring ceased on day 9 of the 21-day foraging trials due to the fact that the change in lighting required to obtain images of tunneling in the field disturbed the foragers within the arena on a daily basis. Since there was very little tunneling after the first week, I opted to discontinue photographing the gallery systems in favor of not creating additional disturbances. There were no significant differences between the area tunneled on either side of the arenas during the 9 days that tunneling was monitored (Fig. 6.9). No dead
Figure 6.9 Average cumulative and daily tunneling amounts on each side of the field arenas
Figure 6.10 Average percent wood loss from wafers on both sides of laboratory control arenas (A), laboratory treated arenas (B), and field arenas. Means from each side with the same letters are not significantly different (within each time period). Means with the same numbers (C) are not significantly different across time periods (within treatment).
termites were seen in the field arenas at any time during the 21-day foraging trials, and there was no evidence of tunnels being backfilled or sealed in response to treated wafers.

6.3.4 Wood Consumption

Laboratory. In the laboratory control arenas, the amount of wood consumed was not significantly different on either side of the arena during the first two weeks, as well as during the last week. Termites consumed significantly more untreated wood than treated wood during the first two weeks in laboratory treated arenas ($F = 9.75; \text{df} = 1, 4; P = 0.0355$), and the amount of feeding that did occur on treated wood was very small and amounted to only slight surface etching (Fig. 6.10). An even smaller amount of feeding occurred during the last week, with no significant difference between sides (Fig. 6.10).

Field. Comparing wood loss between arena sides within each time period, significantly more wood was consumed during the first week on the east side, the side originally containing untreated wood, compared to the west side ($F = 9.69; \text{df} = 1, 4; P = 0.0358$). There were no significant differences in wood loss between sides during the second and third weeks (Fig 6.10). The comparison of wood loss across time periods within treatments showed that the mean percent wood loss from treated wafers was greatest in the second week, but it was only significantly greater when compared to the third week ($F = 5.06; \text{df} = 2, 6; P = 0.0515$) (Fig. 6.10). The greatest amount of untreated wood loss occurred in the first week, and was significantly greater when compared to the third week, but was not significantly greater than the second week ($F = 5.70; \text{df} = 2, 6; P = 0.0411$). There were no significant differences in average wood loss of untreated wafers between the second and third weeks (Fig. 6.10).
6.4 Discussion

The results of this study suggest that avoidance by the Formosan subterranean termite to wood treated with borates in the form of DOT (Tim-bor) at prescribed wood preservation concentrations is not a result of the repellent accumulation of dead foragers in the vicinity of the treated wood. No dead termites were observed in any of the field arenas, and in the laboratory arenas, feeding on treated wood generally ceased before the effects of mortality occurred. Moreover, when mortality did occur in the laboratory, it was usually not in the vicinity of a treated wafer. This was most clearly seen in laboratory treated arena 1 (Fig. 6.3) where initially termites were equally distributed throughout the entire arena and were feeding on both treated and untreated wood. After approximately three days, termites stopped feeding on treated wood, but signs of mortality were not seen until day 5. Once termites started dying, the distribution of termites in the arena shifted dramatically to the east side, or untreated side, even though a majority of the poisoned individuals were also dying on the east side.

A three-day lag time between the beginning of feeding by C. formosanus and the onset of borate’s toxic effects was also observed by Maistrello et al. (2001). Feeding termites wood treated with 0.1571% BAE (considerably less than the current experiment), they reported fed individuals appearing inactive and ailing, and they attributed these symptoms to the toxic action of borate at the cellular level rather than the death of symbiotic intestinal protozoa, which did not fully occur until day 7. It is possible that these initial toxic effects are what first deter feeding, followed a few days later by death when a majority of the symbiotic flagellates have succumbed due to changes in the intestinal microenvironment.
Feeding ceased after a similar amount of time in the second treated laboratory arena, but foragers in this arena fed much more on treated wood during the initial period of feeding. As a result, the onset of mortality was quicker and more severe. In this case, termites moved back onto treated wood because of the accumulation of dead foragers in the untreated area. Because this group of foragers ingested so much borate early in the foraging trial, mortality proceeded at a rate too fast for them to compensate, and they were not able to properly care for dead workers. The dead workers died on and around the untreated wafers, and consequently repelled the surviving termites. An opposite series of events occurred in laboratory treated arena three. Foragers in this arena favored the untreated side and, aside from a small amount of feeding on day 5, did not begin feeding in earnest on treated wafers until almost 11 days into the trial. Consequently, mortality didn’t begin until day 9.

When new wood was added and the locations of treated and untreated wafers were switched, termites returned to feeding at sites where feeding was previously most intense. In treated arenas one and three, these were the sites on the east half of the arena that had previously contained untreated wood. In both of these cases they fed on the newly added treated wood, showing no recognition of the borate treatment. In the field, average consumption of treated wood appeared to be greater during the second week when it was moved to the side that had previously contained untreated wood. The increase was not statistically significant, however, due in part to a very small sample size and variation in feeding rates between the arenas. Nonetheless, these findings suggest that termites did not avoid borate treated wood based on recognition as a result of
previous sublethal exposure. It appears that avoidance is related more to the location of
treated wood than to the chemical treatment itself.

A potential explanation for this could lie in the chemical recruitment system
utilized by termites during foraging. Subterranean termites orient themselves throughout
tunnel systems using trail pheromones deposited by their sternal glands (Luscher and
Muller 1960). Trail pheromones play an important role in directing foragers to feed in
certain areas (Tschinkel and Close 1973). There is evidence that trail pheromones consist
of an ephemeral recruiting component and a more stable orientation component (Hall and
Traniello 1985). The strength of the ephemeral component and subsequent recruitment is
reinforced or diminished by the quality of the food resource (Traniello and Leuthold
2000). Therefore, if the signal is strong, many termites can be induced to feed at a
location, and if the signal is weak, termites may not follow it all. If foragers feeding on
treated wood become sickened and later die after feeding, the ephemeral recruiting
component can undergo a diminishing rate of reinforcement. As fewer termites travel to
the site, there is less pheromone deposited and consequently less foraging traffic until all
that remains is the stable orientation component. Rickli and Leuthhold (1987) found that
for the harvester termite, *Trinivitemes geminatus*, information provided by trail
pheromones was the dominant influence on a termite as it chooses where to go within a
network, but that also the amount of activity by termites within the network played a role.
This may explain why termites in this study stopped visiting sites containing borate
treated wood over time, but resumed feeding on it when it was moved to areas that
previously contained untreated wood.
I thus conclude that initial tunnel formation by a foraging group of Formosan subterranean termites is unaffected by the discovery of borate treated wood. It is unclear however, what the long-term effects of discovered borated treated wood are on a colony as it expands its foraging territory. Additionally, I found that Formosan subterranean termite foragers do not avoid borate treated wood as a result of necrophobic behavior nor from a learned response to borate treated wood. It rather appears that avoidance to the slow acting toxicant may be a behavior mediated by the decreasing amounts of trail pheromone due to mortality within the foraging group. More work on trail pheromone titers and their role in subterranean termite foraging are needed to substantiate this claim.
CHAPTER 7

Conclusion

The work presented here adds insight into the factors that affect subterranean termite foragers as they tunnel through the substrate in search of food. This new information has the potential for application in treatment strategies that utilize non-repellent slow acting termitecides, particularly termite baiting systems. For example, in chapter one it was shown that tunneling activity increases when objects are encountered in the soil, with additional tunnels emanating out from objects in the same general direction as the tunnel that initially intersected them. These findings imply that supplementary bait stations installed in the vicinity of an active station have a greater chance of discovery than stations installed at uniform distances around the perimeter. More work is needed to determine the distance from active stations at which supplementary stations no longer have a spatial advantage in terms of discovery over those that are uniformly distributed.

The finding that open space in the substrate stimulates tunneling and searching behavior also has implications in termite management. Debris such as rocks and other materials in heterogeneous soil may create pockets in the substrate that could lead to increased tunneling by foraging termites. The practice of eliminating these types of anomalies in the soil environment and preventing the accumulation of cellulosic waste underground during building construction has the potential to decrease a structure's likelihood of termite attack. Alternatively, this suggests that aerating the soil or disturbing the soil such that compaction is lessened may increase the probability of station discovery when baiting and the improve the efficacy of type III soil termitecides.
that are partially dependent on active tunneling by a subterranean termite colony's foraging population.

The research presented here also makes it clear that not all colonies of the Formosan subterranean termite are equal with respect to tunneling and foraging rates. Colonies with larger workers are more efficient at tunneling and construct wider tunnels with fewer segments. Furthermore, tunneling rates by colonies in the field can vary significantly. Colonies that are more aggressive tunnelers may therefore be easier to manage through baiting than others. Colony population size and age were identified as potential variables that may influence tunneling rates. However, there are many other aspects, both biotic and abiotic, that may influence a colony's need to forage. The fact that under controlled laboratory conditions no significant differences in tunneling rate were observed between two colonies that showed significant variation in tunneling in the field suggests that laboratory tunneling experiments can reduce variation from the field. Small-scale laboratory experiments on tunneling and foraging can therefore be useful to examine specific termite behaviors that might otherwise be confounded by variation in field conditions when using termites from different colonies.

Finally, evidence was provided that the presence of wood treated with sodium borate to manufacturer recommended retentions does not deter termite tunneling in the surrounding substrate. Furthermore, it was found that foragers that ingested lethal amounts of sodium borate from treated wood did not remain and die in the vicinity of the treated wood. Avoidance of wood treated with this non-repellent wood preservative was therefore shown to not be the result of necrophobic behavior. After the cessation of feeding on borate treated wood, foragers continued to "taste" treated wood as it was
moved to new locations, and they fed on untreated wood in locations previously occupied by treated wood. This leads to the conclusion that termites responded more to the location of the treated wood than to the actual chemical treatment. Additional research is needed to explore the effects of delayed mortality and sublethal exposure on the deposition of trail pheromone, and how these events can affect the distribution of foragers at multiple food locations.

While this dissertation provides new information on the foraging behavior of subterranean termites, it also underscores the fact that very little is known about how subterranean termites locate food and discriminate among food resources within their gallery systems. As the use of non-repellent termiticides increases, there will continue to be a corresponding increase in the need for a greater understanding of these behaviors. In Hawaii, this need could be extended to the foraging behavior of the state’s other less common species of subterranean termite, *Coptotermes vastator* Light. Preliminary studies have shown that this species may have a distinctly different tunneling pattern than *C. formosanus* (Grace et al. 2004). On a broader scale, the implications of some of the research presented in this dissertation lay the groundwork for future studies on the role of pheromones in tunnel formation and the spatial distribution of termites within a colony’s foraging territory. Research on these topics will continue to aid the pest management industry as it seeks to develop new technologies and methods to combat the continued expansion and destructive nature of the Formosan subterranean termite and other subterranean termite species in urban environments.
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