Environmental Influences on Subterranean Termite Foraging Behavior and Bait Acceptance

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ABSTRACT

Reticulitermes spp. was significantly more likely to discover subterranean baits connected by physical guidelines than freestanding baits under both laboratory and field conditions. In the laboratory, subterranean termites built significantly longer tunnels adjacent to guidelines containing cellulose than plastic guidelines. In the field, all guideline materials were equally effective at directing tunneling activity.

Reticulitermes workers were tested to determine their preferred substrate temperature. The preferred range for *Reticulitermes* spp. workers was found to be 18 to 27 ° C. A laboratory bioassay determined that *Reticulitermes* spp. aggregates within thermal shadows. Significantly more *Reticulitermes* workers aggregated within areas that were cooler than the surrounding substrate.

In a choice bioassay, termites consumed significantly more of paper baits treated with fructose, galactose, glucose, raffinose, sucrose, trehalose and uric acid than control baits. Mean consumption was significantly lower for baits treated with arbutin, and several amino acids than for control baits. In the no-choice bioassay, consumption of nutrient treated baits and control baits was not significantly different.

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CHAPTER I: Introduction

Termite baiting systems. The economic impact of subterranean termites in the United States now exceeds $10 billion annually (Su, 2002). One tool that is frequently employed for subterranean termite management is termite baiting systems (Henderson et al., 1998; Su and Scheffrahn, 1998; Potter et al., 2001; Verkirk and Bravery, 2001). Because the active ingredient employed in a termite baiting system remains isolated from the environment in a bait station, unless subterranean termites consume it, these baiting systems have considerable appeal to those concerned with environmental contamination and pesticide exposure (Jones, 1989; French, 1991; Quarles, 1995; Pawson and Gold, 1996; Potter et al., 2001; Verkerk and Bravery, 2001).

All subterranean termite baiting systems work by exploiting the normal foraging and food distribution activities of subterranean termites to deliver toxicants to the entire colony (Olstaff and Gray, 1975; Esenther and Beal, 1979; French, 1991; Su et al., 1995). For a baiting system to provide effective control, subterranean termite foragers must locate and recruit to the bait. The foragers must then consume sufficient amounts of toxicant and transport it back to the nest for distribution throughout the nest population (Esenther and Beal, 1979; Su et al., 1987; Su, 1995a; Grace et al., 1996a; Su and Scheffrahn, 1996).

Ideally insect bait should consist of four components (Klotz et al., 1997):

- An attractant, usually a food or pheromone, which draws the targeted pest to the bait station.

- A carrier, which must be palatable to the target species. This is sometimes also the attractant.

- A toxicant.

- A preservative to assure continued acceptability.

Unfortunately, no practical attractant exists for subterranean termites, nor has it been possible to successfully manipulate termite foraging activity and lead termites to bait stations (Su, 1995; Pawson and Gold, 1996). Because termite bait contains no attractant, two major problems limit the effectiveness of subterranean termite baiting systems. Bait discovery is often slow and, once discovered, baits may not be sufficiently palatable to compete with other food resources. Baits may be abandoned if a more desirable source of food is discovered by the colony or if subterranean termite feeding is disturbed.

Since the time required for subterranean termites to locate a bait station is unpredictable, (Tamashiro et al., 1991; Pawson and Gold, 1996; Henderson et al., 1998; Potter et al., 2001; Ring et al., 2001) consumers become dissatisfied with baiting systems (Potter et al., 2001). The ability to direct foragers to bait stations could decrease the time of discovery and improve the effectiveness of subterranean termite baiting systems.

Physical guidelines. Early field studies (Kofoid, 1934b; King and Spink, 1969; French, 1991) suggested that subterranean termites might follow physical guidelines through the substrate. Pitts-Singer and Forschler (2000) and

Campora and Grace (2001) found that Reticulitermids would follow physical guidelines under laboratory conditions. We compared the ability of guidelines constructed of wood, plastics and cellulosic materials to guide foraging subterranean termites to bait, both in the laboratory and in the field.

Thermal shadows. Several researchers have postulated that subterranean termites might locate food on the soil's surface by sensing thermal shadows (areas of substrate where the soil temperature is detectably different from the temperature of the surrounding soil) beneath surface features (Santos, 1979; Ettershank et al., 1980). During a study of leaf litter degradation, Santos (1979) made the surprising discovery that Reticulitermids found and consumed bags of leaf litter located on the soil surface, but failed to find similar bags covered by six inches of soil. Furthermore, Santos observed that subterranean termites found and consumed boards placed on the soil surface directly over buried litter bags, without finding and consuming the buried litter. Santos postulated that Reticulitermids might sense objects on the soil surface because the soil beneath them was shaded from the sun.

Ettershank et al. (1980) observed that Reticulitermids, tunneling several inches below the soil surface, were able to locate isolated cow pies, in areas of otherwise barren ground. Ettershank's group hypothesized that the termites must either be using chemical plumes in the soil below the potential forage or differences in substrate temperature to locate their food. To test these hypotheses, the researchers buried thin sheets of plastic in the soil a few millimeters below cow pies. Termites, tunneling to the soil surface to consume the manure, produced small holes when they passed through the plastic. By counting these holes, the researchers could determine how many times the cow pie was attacked. Ettershank et al. also constructed artificial plastic cow pies and placed these on the soil surface above sheets of plastic in the same configuration. The researchers reasoned that, if the termites were following chemical plumes to the cow pies, only real cow pies would be attacked. If, on the other hand, the termite foragers were reacting to thermal shadows, both real and artificial cow pies would be attacked. It was discovered that subterranean termite foragers were attracted to both real and artificial cow pies. Ettershank et al. concluded that thermal shadows must be a major cue for foraging subterranean termites. It might, therefore, be possible to direct foraging termites toward bait by manipulating the temperature around in-ground bait stations. Assays were performed to investigate the reaction of subterranean termites to thermal shadows, under laboratory conditions.

Bait matrix palatability. If subterranean termites do not consume a sufficient quantity of bait, a termite baiting system may fail to introduce enough toxicant to significantly impact a nest population. Research suggests that commercial bait matrices may be less palatable to subterranean termites than natural wood products (Pawson and Gold, 1996; Hedlund and Henderson, 1999). In addition, subterranean termites are sensitive to disturbance while feeding. Replacement of a bait matrix in an active station, or replenishment of depleted bait frequently resulted in abandonment of the station by foraging termites (Nutting and Jones, 1990; Su and Scheffrahn, 1996; Su, 2001). One way to improve the performance of termite baiting systems would be to increase the palatability of the bait matrix employed. Improving bait palatability would increase the rate of bait consumption and decrease the probability of bait abandonment. The final assay described in this paper compared the efficacy of nitrogen compounds and sugars as nutrient compounds for *Reticulitermes* spp.

Specific objectives. Reducing the time to bait discovery, increasing the amount of bait consumption and reducing the probability of bait abandonment would enhance the performance of termite baiting systems. Therefore these studies were designed to investigate strategies for directing termite foraging activity in the substrate and increasing the palatability of bait matrices in order to enhance the function of commercial subterranean termite baiting systems.

The specific objectives of this series of experiments were:

- To investigate the influence of physical guidelines on termite foraging behavior both under laboratory conditions and in the field.
- To investigate the influence of thermal shadows on termite aggregation.
- To evaluate the ability of nitrogen compounds and sugars to increase cellulose bait consumption by *Reticulitermes* Spp..

Chapter II: Background and Literature Review

Subterranean termites in historical perspective. The fossilized remains of termites have been documented in 120 million year old rock formations (Krishna, 1992). Termites are of ancient lineage, having evolved approximately 200 million years ago (Krishna, 1992) from cockroach like insects (Prestwick, 1983). Unlike their near relatives, the scavenging cockroaches, termites lead a secretive life (Kofoid, 1934a). The *Reticulitermes* and *Coptotermes* or subterranean termites spend their entire life in complex nests hidden below the surface of the soil or encased in wood (Light, 1934a, b; Wood, 1978b). Wood and other plant tissues comprised largely of cellulose are also the termites' primary source of food (Wood, 1978a; Potter, 1997). The common name, termite, derived from the Latin word *termes*, translates literally as woodworm (Potter, 1997).

Two thousand-year old Chinese documents state that termites were then, as now, an economically important pest of stored goods and wooden structures (Li et al., 1994). Presently, termites are believed to be the largest single cause of lumber decay in tropical areas (Mill, 1991). Thirty pest species of termites are found in North America (Su and Scheffrahn, 1990a). Two genera of termites, *Reticulitermes* and *Coptotermes,* are responsible for most of the structural damage caused by termites in the United States (Su, 2002). Su (2002) reported that the economic impact of subterranean termites in the United States now exceeds $10 billion annually. In the southeastern U.S., the economic impact of termite damage is estimated to be 2-10 times greater than the impact of insect pests of any agricultural commodity (Su and Scheffrahn, 1990a).

Throughout history, humans have sought to protect their possessions from subterranean termites with various poisons. In the early part of the twentieth century, arsenic compounds were popular for treatment of termite infestations (Randall and Doody, 1934 a, b, c). Physical barriers made of copper and lead were also used as a toxic subterranean termite preventative (Brown et al., 1934; Potter, 1997). Later, soil treatments, including trichlorobenzene, were used to prevent subterranean termite attack (Hatfield, 1944; Beard, 1974; Williams, 1977; Su, 2002).

The development of commercially produced liquid termiticides. During the 1920s and 1930s, the first long-term surveys of possible preventive termiticides were initiated by private industry in the United States (Randall and Doody 1934c; Hatfield, 1944). The earliest research into possible soil barrier treatments met with little success. The only compounds identified as effective termiticides were too expensive to be practical (Randall and Doody 1934c). It was not until after World War II that the cyclodienes, a class of chemical compounds identified as highly effective termiticides, became commercially available (Ware, 1999). Soil barrier treatments created by saturating building sites with cyclodienes prior to construction were the standard weapon against structural infestation from the late 1940s until 1988 (Lewis, 1980; Su and Scheffrahn, 1990b Jitunari et al., 1995). The cyclodienes, particularly chlordane, were extremely efficacious and stable in soil, in some cases protecting structures from subterranean termite infestation for several decades (Lenz et al., 1990; Su and Scheffrahn, 1990b; Grace et al., 1993).

Unfortunately, improper application frequently resulted in volatilization of chlordane into the structures built on treated sites (Midwest Research Institute, 1994; Jitinari et al., 1995; Kilburn and Thornton, 1995; Sim et al., 1998; Environmental Protection Agency, 2003; Australian Department of Environment and Heritage, 2004). In addition, the manufacturing practices of Velsicol, the sole licensed producer of chlordane, led to chlordane's widespread introduction into both the atmosphere and groundwater (Midwest Research Institute, 1994; US Geological Survey, 1996; Environmental Protection Agency (EPA), 2003).

Because of cyclodiene residual longevity, questions were raised about the environmental impact of these chemicals (Lewis, 1980; Su and Scheffrahn, 1990a; Mill, 1991; Tamashiro et al., 1991; Wood and Pierce, 1991; Singh et al., 1992; Calder et al., 1993; Stevens et al., 1993). Research demonstrated that long term exposure to organochlorines, like the cyclodienes, resulted in biomagnification (EPA, 1998) and accretion in the fatty tissues of humans (Lewis, 1980; Stevens et al., 1993; Jitunari et al., 1995; Kilburn and Thornton, 1995; EPA, 1998; Nasir et al., 1998; Sim et al., 1998) and other organisms (Lewis, 1980; Cassidy et al., 1994; EPA, 1998; Walker and Newton, 1998; Stansley and Roscoe, 1999). In 1973, Velsicol Chemical Corporation, the licensed producer of chlordane, funded a study performed by the International Research and Development Corporation (IRDC) to determine chlordane's mammalian toxicity. IRDC reported that mice given oral doses of 25- and 50-ppm of technical chlordane daily for 18 months had decreased survival, relative to controls. EPA-sponsored examination of mouse liver histological slides found a statistically significant increase of carcinomas in groups of mice exposed to 25- and 50-ppm doses (EPA, 1998). As a result of this study, most agricultural uses of cyclodienes in the U.S. were canceled by 1980 (Ware, 1991; EPA, 1998). In 1988, use of chlordane and heptachlor, as termiticides, was canceled by mutual agreement of the EPA and Velsicol Corporation (Robertson and Su, 1995a, b; EPA, 1998; Ware, 2000).

For the next decade, the only termiticides available for use as soil barrier treatments were chlorpyrifos (an organophosphate) and several pyrethroids. The residual activity of chlorpyrifos was significantly shorter than that of the cyclodienes (Lenz et al., 1990; Grace et al., 1993). In addition, the toxicity of chlorpyrifos to subterranean termites was significantly affected by variations in the clay and cellulose content of treated soil (Smith and Rust, 1993). As a result of the Food Quality Protection Act of 1996, EPA revised its risk assessment of chlorpyrifos and, in the year 2000, joint agreement of the EPA and the registered manufacturers of chlorpyrifos canceled chlorpyrifos' use as a soil barrier treatment against subterranean termites (EPA, 2000).

The pyrethroids are more durable than chlorpyrifos, but less stable in the soil than the cyclodienes (Lenz et al., 1990; Su and Scheffrahn, 1990b; Oka et al., 1995; Pawson and Gold, 1996). Soil barriers composed of pyrethroids are more likely to fail than barriers composed of cyclodienes or chlorpyrifos (Lenz et al., 1990; Su and Scheffrahn, 1990b; Su et al., 1993b; Forschler, 1994; Kard,

1999) because pyrethroids are repellant to subterranean termites (Su and Scheffrahn, 1990b; Rust and Smith, 1993; Su et al., 1993b). Subterranean termite foragers are able to detect and avoid repellant termiticides so areas treated with pyrethroids are rarely contacted. The subterranean termites' ability to detect chemical barriers allows termite foragers to follow the edge of the pyrethroid treated area until they find a gap in the treatment (Su et al., 1982; Su and Scheffrahn, 1990b; Rust and Smith, 1993; Forschler, 1994). Thus, gaps in pyrethroid applications may actually funnel foragers toward the structures they are intended to protect (Forschler, 1994; Kuriachan and Gold, 1998).

The inevitability of gaps in soil termiticide barriers is a major limitation to the efficacy of repellant liquid termiticides (Forschler, 1994; Kuriachan and Gold, 1998). Gaps may exist in a soil termiticide treatment for a number of reasons. Pre-construction treatments often contain gaps due to imperfect initial application or physical disturbance of the soil after application (Su and Scheffrahn, 1990a, 1998; Koehler et al., 2000). When an existing structure becomes infested and requires a remedial termiticide application, it is difficult to create a continuous horizontal barrier of liquid termiticide beneath the structure (Su and Scheffrahn, 1990a, 1998; Koehler et al., 2000). Finally, all termiticides degrade over time. An ageing soil treatment, applied below the foundation before a structure was built, is inaccessible after construction and cannot be reapplied (Su and Scheffrahn, 1990a; Su, 1997; Koehler et al., 2000).

Beginning in the year 2000, several new nonrepellant soil termiticides appeared on the market: fipronil, a phenyl pyrazole (Aventis Corp., 2001 a, b);

imidacloprid, a chloronicotinyl (Bayer Corp., 2000a); and chlorfenapyr, a pyrrole (BASF Corp., 2001). Currently, these three nonrepellant termiticides and the pyrethroids are the only formulations available for use as structural soil barrier treatments (Wagner et al., 2003). Nonrepellant termiticides are an improvement on the pyrethroids because subterranean termites cannot detect gaps in the treatment and use them to gain access to structures (Potter and Hillery, 2001). Subterranean termites are unable to detect the termiticide and do not avoid soil that has been treated with them (Kuriachan and Gold, 1998).

Disadvantages of currently available soil termiticides. Unfortunately, none of the new nonrepellant soil termiticides possesses the combined qualities of economy, efficacy, and longevity that were characteristic of the organochlorines. All three nonrepellant termiticides are significantly more expensive than the cyclodienes and none of them have exhibited the prolonged residual activity (up to six decades) of chlordane (Su and Scheffrahn, 1998; Wagner et al., 2003). None of the nonrepellant termiticides currently labeled for use against subterranean termites can guarantee even a decade of security against termite infestation, when applied as a soil barrier prior to construction (Wagner et al., 2003).

Another drawback to the use of liquid termiticide is the continued concern over environmental impact (Beard, 1974; Ostaff and Gray, 1975; Williams, 1977; Lewis, 1980; Jones, 1989; Su and Scheffrahn, 1990a; French, 1991; Wood and Pierce, 1991; Peyton et al., 1995; Pawson and Gold, 1996; Sim et al., 1998; Potter et al., 2001; Potter and Hillery, 2001; Verkerk and Bravery, 2001). All liquid termiticides currently labeled for use in the United States exhibit lower mammalian toxicity than the organochlorines, however the creation of liquid termiticide barriers still requires the introduction of large volumes of highly residual insecticides into the soil (Esenther and Beal, 1979; Su and Scheffrahn, 1990a, 1998; Potter and Hillery, 2001). Legislation produced in response to environmental concerns now places serious constraints on the use of liquid termiticides. EPA Pesticide Regulation Notice 96-7 article G specifies strict limitations on the application of liquid termiticides in areas adjacent to wells, cisterns and other bodies of water to avoid groundwater contamination.

Integrated pest management. Concerns over human health and environmental pollution by liquid termiticides, and the limitations of both the nonrepellant termiticides and pyrethroids have created a need for alternative methods of subterranean termite control. A number of novel termite control products, including physical barriers, wood treatments and baiting systems, are now commercially available. Pest management professionals can choose from an array of mitigation techniques to create an integrated pest management (IPM) program designed to address a specific termite control challenge (Quarles, 1995; Su and Scheffrahn, 1998; Campora and Grace, 2001; Potter et al., 2001; Verkirk and Bravery, 2001; Grace and Su, 2001; Lee, 2002; Su, 2002; Wagner et al., 2003).

Shelley (2000) defined integrated pest management as:

"…a pest management strategy that focuses on long-term prevention or suppression of pest problems through a combination of techniques such as monitoring for pest presence and establishing treatment threshold levels, using non-chemical practices to make the habitat less conducive to pest development, improving sanitation, and employing mechanical and physical controls. Pesticides that pose the least possible hazard and are effective in a manner that minimizes risks to people, property, and the environment, are used only after careful monitoring indicates they are needed according to pre-established guidelines and treatment thresholds".

The concept of IPM first became popular in the early 1960s. At that time, the focus of the IPM movement was the reduction of pesticide use in agriculture. Later, the same key principles (long-term prevention, multiple control strategies and minimal pesticide use) were incorporated into structural pest management programs. Beginning in the 1990s, many public organizations, including schools, military bases, and government facilities, were mandated to develop IPM programs to reduce human exposure to toxic chemicals and minimize the release of pesticides into the environment (International Pest Management Institute, 2003). One tool that is frequently employed as a part of an IPM program for subterranean termite management is the termite baiting system (Henderson et al., 1998; Su and Scheffrahn, 1998; Potter et al., 2001; Verkirk and Bravery, 2001).

The development of subterranean termite baiting systems. Prior to the development of liquid termiticides, toxic baits had been used extensively to control a variety of urban pests (Barber, 1920; Robertson and Su, 1995a). Although baits had not been successfully deployed against subterranean termites in the United States, baits had been successfully used to control subterranean termites in Australia as early as the 1930s (Randall and Doody, 1934b). In North

America, subterranean termite bait research began as early as 1934 but, with the introduction of cyclodienes, interest in baiting technology waned (Esenther and Beal, 1979; Robertson and Su, 1995). However, during the 1970s, the growing environmental concerns related to persistent soil poisons led to a renewed interest in the use of termite baits (Olstaff and Gray, 1975; Esenther and Beal, 1979; Jones, 1989; Tamashiro et al., 1991).

In 1994, the Sentricon™ system, a product of DowElanco, appeared on the market. Sentricon™ was the first commercially available subterranean termite baiting system (Quarles, 1995; Robertson and Su, 1995; Su et al., 1995). Baiting technology was a major breakthrough. Baits quickly captured a significant portion of the termite control market (Pawson and Gold, 1996; Su and Scheffrahn, 1998; Potter et al., 2001). Sentricon™ was followed shortly by several other baiting systems, due to its commercial success (Quarles, 1995; Pawson and Gold, 1996; Su and Scheffrahn, 1998).

Termite baiting systems have great appeal for those individuals concerned with environmental contamination and pesticide exposure (Jones, 1989; French, 1991; Quarles, 1995; Pawson and Gold, 1996; Potter et al., 2001; Verkerk and Bravery, 2001). Termite baits are also especially suitable for use at sites in close proximity to water or in areas that are environmentally sensitive (Robertson and Su, 1995; Su, 1997; Su and Scheffrahn, 1998).

All subterranean termite baiting systems exploit the natural foraging and food distribution activities of subterranean termites to deliver toxicants to the entire colony (Olstaff and Gray, 1975; Esenther and Beal, 1979; French, 1991; Su et al., 1995). In the Sentricon System™, plastic stations containing a block of wood (placebo) are installed in the ground around the perimeter of a structure. When termites are found in a station, the placebo is replaced with a bait matrix containing hexaflumuron, a chitin synthesis inhibitor for insects that is otherwise environmentally benign (Su, 1995a). The subterranean termite foragers must consume the bait and carry the hexaflumuron back to the nest for distribution throughout the colony via trophallaxis (the exchange of alimentary fluid among colony members; Su et al., 1995; Grace et al., 1996a). Commercial baiting systems differ, in that the original matrix presented in the station at the time of installation may or may not contain an active ingredient (FMC Corp., 1999; BASF Corp., 2001). However, all currently available commercial baiting systems rely, at least to some degree, on termite trophallactic behavior to deliver their toxicant to additional members of the colony.

Persistent problems with existing subterranean termite baiting systems. While exploiting subterranean termite behavior to control their populations is an excellent idea in theory, the elimination process employed by termite baiting systems is cumbersome. For a baiting system to work, subterranean termite foragers must locate and recruit others to the bait, consume a sufficient amount of toxicant to affect the colony and transport it back to the nest for distribution throughout the population at levels sufficient to affect nest activity (Esenther and Beal, 1979; Su et al., 1987; Su, 1995a; Grace et al., 1996a; Su and Scheffrahn, 1996). Unfortunately, subterranean termite feeding behavior is variable. Bait acceptance and the rate of feeding by individual subterranean termites are difficult to predict (Forschler, 1996). Pawson and Gold (1996) found that ordinary wooden stakes were attacked significantly more often than commercial termite bait stations in the field and theorized that the toxicant contained in commercial stations might be unpalatable to subterranean termites. Thorne et al. (1996) found that subterranean termite movement between wooden monitors was unpredictable and erratic. Termites fed sporadically on a monitor and then abandoned it for no apparent reason. In addition, subterranean termites are sensitive to disturbance while feeding. Replacement of the bait matrix in an active station, or replenishment of a depleted bait frequently resulted in abandonment of the station by foraging termites (Nutting and Jones, 1990; Su and Scheffrahn, 1996; Su, 2001). Finally, if subterranean termites do not consume a sufficient quantity of bait, they may fail to introduce enough toxicant to impact a colony. Another persistent problem with baiting systems, as a subterranean termite control strategy, has been the inconsistency of termite recruitment to baits (French, 1991; Pawson and Gold, 1996; Henderson et al., 1998; Potter et al., 2001; Verkirk and Bravery, 2001). Ideally insect baits should consist of four components (Klotz et al., 1997):

- An attractant, usually a food or pheromone, which draws the targeted pest to the bait station.

- A carrier, which must be palatable to the target species. This is sometimes also the attractant.

- A toxicant.

- A preservative to assure continued acceptability.

Unfortunately, no practical attractant exists for subterranean termites. Subterranean termites are incapable of detecting volatiles emanating from food items, through even a few millimeters of substrate (Campora and Grace 2001; Cornelius and Osbrink, 2001; Puche and Su, 2001b). If subterranean termites do not use olfactory cues to locate food, it is unlikely that traditional attractants (food and pheromones) will be effective in attracting termites to in-ground bait stations. Because it is impossible, at present, to successfully manipulate termite foraging activity and lead termites to bait stations (Su, 1995; Pawson and Gold, 1996), the time required for subterranean termites to locate a bait station is unpredictable (Tamashiro et al., 1991; Pawson and Gold, 1996; Henderson et al., 1998; Potter et al., 2001; Ring et al., 2001). Therefore, bait stations must be monitored frequently to determine whether subterranean termites have attacked them. This process is labor intensive and expensive (Quarles, 1995; Potter et al. 2001; Verkirk and Bravery, 2001). During the period prior to subterranean termites discovering the bait stations, homeowners often become dissatisfied with baiting systems (Potter et al., 2001).

Improving the effectiveness of subterranean termite baiting systems. Reducing the time to bait discovery, increasing the amount of bait consumption and/or reducing the probability of bait abandonment would enhance the performance of termite baiting systems. In order to properly address the problem of bait system improvement, we must first understand termite foraging organization.

Subterranean termite foraging strategy. The problem of erratic recruitment to bait is directly related to the normal foraging strategy of subterranean termites. Subterranean termites, like all foraging insects follow a hierarchy of behaviors when searching for food. Initially, an insect searches for a food patch (the appropriate habitat in which to locate food). Once a likely foraging area is located, insects search within it for potential resources. When a nutritional resource is located, it must be examined and recognized as potentially edible. Finally, the food must be accepted and consumed (Matthews and Matthews, 1978).

For the Isoptera, the search for a food patch is truncated. Primary reproductives choose the initial nesting site at the culmination of the nuptial flight. The foraging area for the colony is established based on the nest location, although the search for food may extend out many meters from that center (King and Spink, 1969; Su and Scheffrahn, 1988; Lys and Leuthhold, 1991; Su et al., 1993a). For subterranean termites, the second stage of foraging, the search for food within the patch consists of exploratory tunneling around foci of the nest complex (Robson et al., 1995; Reinhard et al., 1997). Food resources, once located, then are examined (Goldberg, 1973b; Lys and Leuthhold, 1991; Hedlund and Henderson, 1999; Campora and Grace, 2001). If the food is accepted and consumed, the forager lays a pheromone trail back to the nest. A primary gallery then is constructed around this recruitment trail (King and Spink, 1969; Goldberg, 1973b; Lys and Leuthhold, 1991; Reinhard et al., 1997).

It is incorrect to characterize the subterranean termite's search for food within the patch as random activity (Robson et al., 1995; Reinhard et al., 1997). Several economically important species of Rhinotermitidae employ a strategy that minimizes redundant foraging (Robson et al., 1995; Reinhard et al., 1997; Hedlund and Henderson, 1999; Fig. 2.1). In the laboratory, habitat exploration by subterranean termite begins with a division of the foraging area around the starting point into equal units. Workers build long straight tunnels that radiate out in a star-like pattern from the point of origin, (usually the point at which the termites were introduced into the arena) (Becker, 1972; Campora and Grace, 2001; Cornelius and Osbrink, 2001; Puche and Su, 2001b). The search radius continues to expand outward until food is found or an insurmountable barrier is encountered (Robson et al., 1995; Campora and Grace, 2001; Puche and Su, 2001a). If food is located an expanded gallery or 'primary tunnel' is constructed along the recruitment trail (Fig. 2.1).

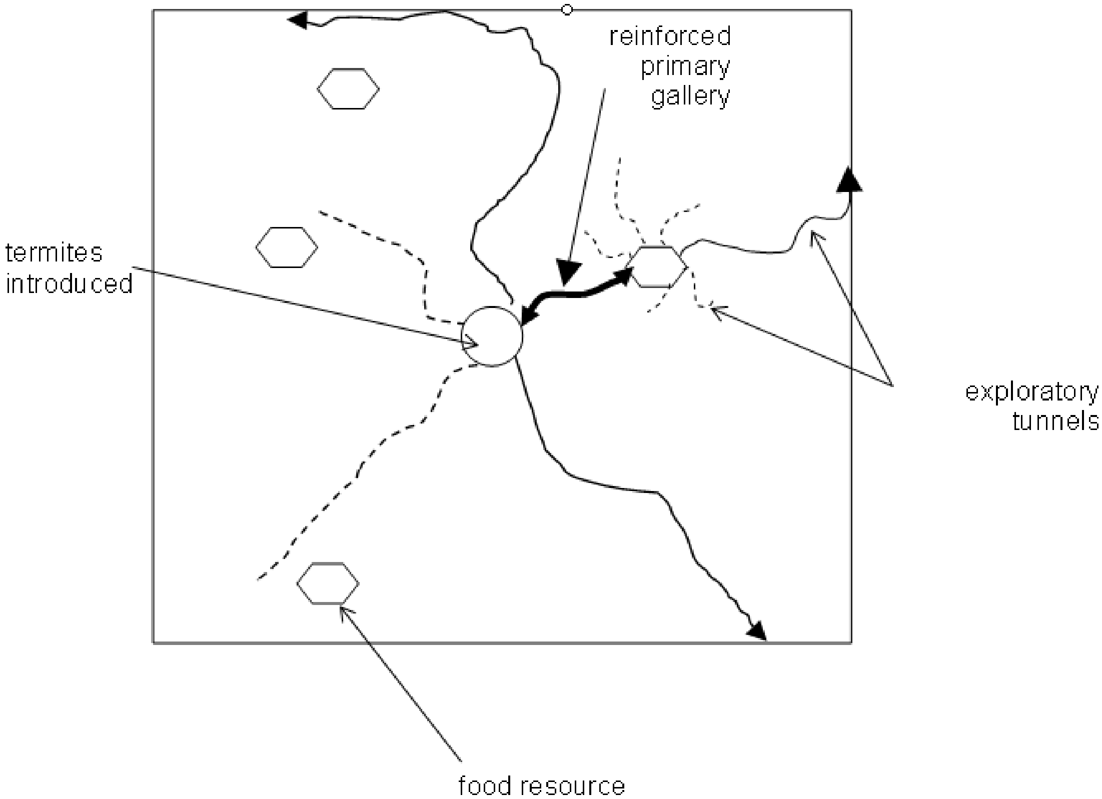


Figure 2.1. Typical configuration of galleries created by termites exploring a laboratory arena. Broken lines indicate abandoned tunnels.

After the food is depleted, exploration of the environment begins again with the current food resource as the new center of activity (Reinhard et al., 1997; Campora and Grace, 2001; Puche and Su, 2001a). By initiating a new search, remote from the original starting location, the probability of searching the same area twice is reduced. However, if the distance between the center point of the original search and the new food resource is great, a substantial area between the two exploration centers may remain uninvestigated.

Campora and Grace (2001) found that the exploratory tunnels generated around secondary foraging sites (blocks of wood) within a laboratory arena composed of sand sandwiched between two layers of Plexiglas, remained relatively short. The majority of these tunnels were quickly abandoned if no food was discovered nearby. The longer the primary gallery between the first food source and the secondary foraging site, the more likely that area adjacent to the primary gallery will remain unexplored. Thus, the likelihood of a food resource being discovered is a function of its proximity to previously exploited resources (Hedland and Henderson, 1999; Campora and Grace, 2001). While organized foraging by subterranean termites has now been well documented in the laboratory, field studies are needed to confirm the fact that *Reticulitermes* spp. behaves the same way in the field, where numerous environmental influences can interact.

Surprisingly little is known about the life history of Reticulitermids. Subterranean termites are rarely seen because *Reticulitermes* spp. chitinous integument is extremely thin and fragile, except on the mouthparts (Light, 1934a; Krishna, 1992). If exposed outside of the nest, Reticulitermids are subject to desiccation, (Kofoid, 1934b; Holway, 1941; Collins, 1969, 1991; King and Spink, 1969; Becker, 1972; Williams, 1977; Puche and Su, 2003) and vulnerable to attack by predators, like ants (Cornelius and Grace, 1994; Cornelius et al., 1995). To avoid predators and maintain high relative humidity, subterranean termites spend their entire lives encased in galleries composed of wood or a mixture of soil, saliva and excrement known as carton material (Light, 1934a, b; Wood, 1978b; Krishna, 1992). It is because of the termites' secretive lifestyle that structures attacked by subterranean termites may suffer severe damage before the insects are discovered.

Because of the cryptic nature of subterranean termite existence, field studies on the foraging behavior of the Rhinotermitidae are rare (Haverty et al., 1974; Campora and Grace, 2001; Su, 2001b). However, there is evidence that wild populations adhere to the same foraging practices observed in the laboratory studies described above. Williams (1977) noted that species of *Reticulitermes,* including *R. flavipes*, build dense networks of exploratory tunnels radiating from existing food resources. King and Spink (1969) reported that an excavated *C. formosanus* nest was “surrounded by a succession of consumed food supplies”, connected by large, straight tunnels or galleries lined with carton material. The nest consisted of three main galleries and numerous side galleries totaling roughly 1,900 feet in length. King and Spink (1969) postulated that the main galleries associated with the central nest were expanded along a course defined by successive food resources.

Food sources may be bypassed, especially as termite galleries expand further and further from the main nest in a relatively linear fashion, leaving areas between the main galleries, used to transport food back to the colony, unexplored (Hedlund and Henderson, 1999). In a Louisiana study, conducted at the site of a long-term *C. formosanus* infestation, 428 wooden stakes were placed adjacent to areas of termite activity. At the end of the 13-month study only 7% of the stakes had been attacked (Henderson et al., 1998). In a similar study carried out in Hawaii, Tamashiro et al. (1991) placed wooden stakes surrounded by corrugated cardboard, adjacent to known centers of *C. formosanus* activity. Termites recruited to only 50% of these controls during a four-year serial and all infestations were initiated during the first two years. Tamashiro et al. (1991) suggested that stakes that were “missed” on the first “exploratory run” by the termites remained undiscovered.

The impact of subterranean termite foraging strategy on commercial baiting systems. If resources that are located far from other termite food resources are less likely to be located by subterranean termites, it would have serious implications for the expected efficacy of commercial termite baiting systems. All termite bait stations are a relatively small presence in the environment and are typically installed at ten-foot intervals (Bayer, 2000b; Ensystex, 2001). One way to improve the performance of termite baiting systems would be to locate stations in areas where subterranean termites are likely to forage, or to enhance the substrate around the stations in a way that would encourage subterranean termites to tunnel there. In order to predict where subterranean termites are likely to tunnel or to encourage them to explore certain areas, we must understand the environmental and behavioral factors that influence termite movement within the substrate.

Environmental and behavioral factors influencing subterranean termite feeding. There are a number of factors that affect when and where subterranean termites feed and how much of a food resource they will consume before resuming foraging activity. *Reticulitermes* spp. feeds preferentially on certain wood species such as sugar maple, slash pine, and Douglas fir (Smythe and Carter, 1970; Waller, 1988) and on wood that has been decayed by certain fungal species, including *Gloeophyllum trabeum* (Persoon: Fries), *Marasmiellus* *troyanus* (Murrill) and *Phanerochaete chrysosporium* Burdsall (Ambergey, 1979; Cornelius et al., 2002). However, preferred food resources are not always available. A field study by Pawson and Gold (1996) evaluated the probability of subterranean termite attack on different bait matrices and wooden stakes within the same plot. The authors postulated that subterranean termites will consume less preferred items when they are the only food available, but will continue to search for more desirable forage while the existing food resource is under exploitation. Depletion of food resources is also known to stimulate renewed subterranean termite foraging activity (Delaplane and La Fage, 1989).

When the activity of a subterranean termite colony is focused on investigating and exploiting a preferred food resource, exploratory tunnels are abandoned and may even be backfilled (King and Spink, 1969; Campora and Grace, 2001). Therefore, the discovery of a large food resource, like a wooden structure, could greatly reduce exploratory foraging by a colony. Hedlund and Henderson (1999) found that foraging activity decreases in the area of large established food resources. In fact, they found evidence that the number of workers allocated to exploit a food resource was related to the amount of food available for exploitation.

The allocation of an appropriate work force to attack a food resource may be a behavioral adaptation by termites to facilitate resource exploitation, while minimizing the expenditure of energy. If too many termites aggregate at a small food resource, individuals might not be able to consume sufficient food to compensate for the energy used in traveling to and from the feeding site.

However, because wood is tough and resistant to structural degradation, efficient exploitation of wood by termites requires the joint effort of many individuals.

Cluster feeding. In a laboratory study, Delaplane and La Fage (1989) found that *C. formosanus* did not equally exploit a group of equivalent wood blocks but foraged as a group on one block, ignoring the others. This phenomenon is known as cluster feeding (Kaib, 2001). Kaib (2001) found that termite species representative of all major families, including the Rhinotermitidae; form feeding clusters to exploit nutritional resources (Kaib, 2001; Reinhard et al., 2002). Cluster feeding is believed to be an adaptive response to the intractable nature of the termites' chief food, cellulose. Simultaneous feeding by multiple individuals facilitates the mechanical breakup of the food item, humidifies the food through saliva deposition (Kaib, 2001; Reinhard et al., 2002), and stimulates increased feeding through the release of hydroquinone. The labial glands produce hydroquinone, which serves a phagostimulant for the order Isoptera (Reinhard et al., 2002).

Nutrient compounds. In addition to hydroquinone, a number of other chemicals are known to influence subterranean termite feeding behavior. Some species of wood contain water-soluble chemicals that act as subterranean termite phagostimulants (Doi et al., 1999). Chen and Henderson (1996) found that the amino acids: d-aspartic acid, l-glutamic acid, l-proline, l-lysine and l-isoleucine, also acted as phagostimulants, significantly increasing paper consumption in *C. formosanus.* Waller and Curtis (2003) found that *R. virginicus* and *R. flavipes* consumed significantly more filter paper treated with 1% or 3% solutions of glucose, xylose and sucrose than water-treated filter paper.

Attractants. While subterranean termites use olfactory or gustatory cues to assess food resources, apparently subterranean termites do not detect or respond to volatiles produced by sound wood buried in the substrate. Several studies have documented that *R. flavipes* was unable to detect sound wood buried in sand at distances as short as 2.5 mm (Campora and Grace 2001; Cornelius and Osbrink, 2001; Puche and Su, 2001b). Subterranean termites must therefore use environmental cues other than wood volatiles to direct their foraging activity.

Pheromones. The most obvious influence on subterranean termite foraging behavior is the presence of a trail pheromone. It is well established that subterranean termites produce trail pheromones (Tai et al., 1969; Howard et al., 1976; Prestwick, 1983; Runcie, 1987; Grace, 1991; Wobst et al., 1999). *Coptotermes formosanus* and *Reticulitermes spp.* both produce a trail pheromone consisting of a straight chain dodecatrienol and other species-specific components (Kaib et al., 1982). This alcohol is produced in the epidermal gland located on the fourth abdominal sternite (Matsumura et al., 1972; Kaib et al., 1982; Grace, 1991). Subterranean termite trail pheromones can induce both intraspecific and interspecific activity within the Rhinotermitidae (Wobst et al., 1999). Interestingly, dodecatrienols are also natural chemical components of fungus-decayed wood (Watanabe and Cassida, 1963; Matsumura et al., 1969; Grace, 1991).

Subterranean termites are attracted to various wood-rot fungi, most notably *Gloeophyllum trabeum* or brown wood rot fungus (Amburgey and Smythe, 1977; Amburgey, 1979; Esenther and Beal, 1979). Cornelius et al. (2002) also found that the tunneling activity of *C. formosanus* and *R. flavipes* significantly increased in sand treated with an extract of sawdust infected with *G. trabeum, Phanerochaete chrysosporiun* Burdsall or *Marasmiellus troyanus* (Murrill).

Foraging activity and vegetative cover. Subterranean termite foraging behavior is influenced by environmental factors other than chemical cues. The type and density of vegetative cover in an area affects subterranean termite foraging. Whitford (1999) found that subterranean termite foragers were more common in areas populated with mesquite and creosote bush, which formed part of their natural forage, than in adjacent grasslands that did not support a population of preferred food plants. Additional studies found that termites are more likely to be located in areas with vegetative cover than open areas (Light, 1934c; King and Spink, 1969; Jones et al., 1987). For example, French (1991) noted that Australian *Coptotermes* colonies were frequently located in or closely associated with living trees and that bait stations located in the area of trees were recruited to preferentially over stations located in the open. Kofoid (1934b) stated that Reticulitermids were especially given to inhabiting areas containing the dead roots of trees and plants.

There are a number of reasons why termites may prefer to tunnel beneath vegetation, including abundance of forage, increased humidity, structural protection and thermal shadows. Obviously, there are likely to be more termite food resources available in areas with abundant vegetation than in areas of barren substrate. Subterranean termites have high moisture requirements and close proximity to wood, either living or decayed, might tend to maintain the high relative humidity in their environment (Kofoid, 1934b; Holway, 1941; King and Spink, 1969; Becker, 1972; Williams, 1977; Puche and Su, 2003). Tunneling through roots or logs provides structural protection against flooding, tunnel collapse in unstable substrates, and predators. Building a tunnel directly beneath a root allows subterranean termites to exploit voids in the soil created around the root as it grows or decays (Mun and Whitford, 1998). Exploitation of such voids could provide easier passage through layers of substrate that might otherwise be resistant to tunneling. Following subterranean arboreal guidelines could, moreover, allow the termites to exploit the natural growth patterns of a preferred food plant and enhance foraging success. For example, a root is likely to lead to fallen wood under the canopy of a tree.

Substrate temperature. Finally, vegetative cover creates thermal shadows (areas of substrate where the soil temperature is detectably different from the temperature of the surrounding soil). Temperature regulation might play a role in subterranean termite preference for areas with vegetative cover. Smith and Rust (1994) documented a preferred temperature range for *Reticulitermes* *hesperus* Banks, a desert species, and suggested that thermal shadows under vegetation might provide refuge from high temperatures.

Environmental temperature is known to affect subterranean termite foraging activity. In general, subterranean termite activity increases as soil temperature increases, within the critical temperature range for termite survival (Smythe and Williams, 1972; Delaplane et al., 1991). Rust et al. (1996) found that soil temperature dramatically affected feeding by *R. hesperus,* resulting in predictable seasonal patterns of foraging activity. Kofoid (1934b) also reported that seasonal fluctuations in the termite species foraging in an arid mountainous region of California were directly correlated to air temperature. In a Louisiana field study, Delaplane et al. (1991) found that the feeding rate of *C. formosanus* was positively correlated with temperature, but leveled off when temperatures became too high. The tunneling and feeding behavior of *R. flavipes* was also found to be directly impacted by variations in substrate temperature (Strack and Myles, 1997).

Soil moisture content. Soil moisture content is another environmental factor that has been demonstrated to impact subterranean termite foraging activity. Termites are prone to desiccation (Kofoid 1934b; Collins, 1969; Rudolph, 1990). Access to water is an important limiting factor in the survival of subterranean termites (Kofoid, 1934b; Light, 1934b; Collins, 1969; Rudolph, 1990; Forschler and Henderson, 1995). Soil moisture content and ambient atmospheric humidity, a related factor for underground species, are also important limiting factors in subterranean termite tunneling activity. Su and

Puche (2003) found that both *Reticulitermes* spp. and *C. formosanus* tunneled significantly more in areas of sand with a higher moisture content in a two dimensional arena. In the field, King and Spink (1969) reported vertical tunnels connecting primary galleries in a *C. formosanus* nest to ground water. Vertical tunnels are more common in sand than in clay (King and Spink, 1969). Soils with high clay content have a greater capacity to retain moisture than soils composed primarily of sand (Brady and Weil, 1999), suggesting that part of the function of such tunnels may be regulation of humidity within the nest.

Thomas Powell of the University of Florida (personal communication) studied the relationship between soil type and moisture content and *R. flavipes* tunneling activity under laboratory conditions. He found that the soil moisture level needed to induce termite tunneling varied with soil type. In general, 10% - 30% soil moisture produced the most tunneling activity. Higher moisture levels of 20% - 30% were necessary to induce tunneling through Georgia piedmont clay. Moisture content above 30% resulted in saturation of the clay. Moisture levels of 10% - 20% produced active tunneling in fine Bonneau sand, and moisture levels of over 20% caused saturation. Soils to which no water was added (<5%) produced no subterranean termite tunneling activity.

Substrate composition. Subterranean termites are also known to prefer foraging in certain types of substrate. King and Spink (1969) found that *R. flavipes* tunneled preferentially through clay over sand, in a field situation where both soil types were present. *Coptotermes formosanus,* active in the same area, preferred sand to clay. In the same study, *C. formosanus* was frequently observed to build tunnels following the junction between clay and sand layers of soil in mixed strata, using the seam as a guideline for tunneling activity, and possibly exploiting gaps formed at the fissure.

Some soils are practically impenetrable to subterranean termites. Particle size is an important factor in substrate penetration for subterranean termites and layers of sieved sand, gravel or even glass have been used as physical barriers to termite infestation (French, 1991; Tamashiro et al., 1991; Grace et al., 1996b). However, soil particle size that inhibits subterranean termite tunneling varies between termite species (Su and Scheffrahn, 1992).

Summary. Subterranean termites are the most economically significant structural pest in North America. In the past, the creation of barriers in the soil through the application of liquid termiticides was the primary technique used to exclude subterranean termites from structures. Due to public health and environmental concerns, the most effective chemicals formerly used in soil barrier treatments, the cyclodienes, are no longer available for use as termiticides. Unfortunately, none of the soil termiticides currently labeled for use as soil barriers possesses the combined qualities of economy, efficacy, and longevity that were characteristic of the cyclodienes. As a result, a number of novel termite control products, including the subterranean termite baiting system, are now commercially available. Termite baiting systems are especially suitable for use near human structures or in areas that are environmentally sensitive because the toxicant remains contained in the bait station until it is actually consumed by termites. In addition the pesticides employed in termite baiting systems have very low mammalian toxicity.

Unfortunately, there is, at present, no commercially available termite attractant. Termites must discover baiting systems by chance. The time required for termites to find a bait station is unpredictable. Bait stations are also frequently deserted.

The factors affecting *Reticulitermes* spp. foraging and feeding behaviors are complex. It has been demonstrated that termite tunneling increases in the presence of certain types of vegetation and soil chemicals. Termites are known to follow physical guidelines, like roots, through the soil and there is anecdotal evidence that they may follow inorganic guidelines as well. In addition, termites are believed to locate food on the soil surface by detecting differences in substrate temperature. It may be possible to use guidelines to direct *Reticulitermes* spp. toward a bait station. It is also possible that saturation of the soil around a bait station with attractive chemicals or manipulation of the soil temperature might attract Reticulitermids to bait.

It has been demonstrated that termites feed preferentially on certain foods and there is evidence that *Reticulitermes* spp. will desert a known food resource for one that is larger or more palatable. By identifying nutrients that are highly palatable to Reticulitermids, we may be able to minimize the possibility of bait dissertation and increase bait consumption.

Since the baiting system has the potential to be a safe and effective termite control strategy, it is important to investigate ways to improve its performance. To make subterranean termite baiting systems more efficient, we must understand subterranean termite foraging behavior. Very little applied research has been done to investigate which environmental cues are most important to foraging subterranean termites and how environmental cues can be exploited to direct termites toward in-ground bait stations. The series of experiments described in this dissertation were designed to investigate how known termite behaviors might be used to improve the function of subterranean termite baiting systems.

Chapter III: Influence of Physical Guidelines on Subterranean Termite tunneling, Bait discovery and Consumption

Introduction

Subterranean termites (Isoptera: Rhinotermitidae) cause billions of dollars in structural damage annually around the world (Su and Scheffrahn, 1990a). Because of the economic significance of these termites, numerous monitoring and control tactics have been evaluated for their ability to detect and suppress termite activity (Su and Scheffrahn, 1990a, b; Quarles, 1995; Pawson and Gold, 1996; Henderson, et al., 1998; Su et al., 2001; Grace and Su, 2001; Verkerk and Bravery, 2001). Arguably, the two most widely used methods of subterranean termite control in the United States are soil applications of liquid termiticide and termite baiting systems (Potter, 1997). Although liquid termiticide soil applications have been the predominant method of subterranean termite control for the last 50 years (Thorne and Forschler, 1998), termite bait systems have become an important competitor in the termite market over the last decade (Potter 1997).

Termite bait systems have been a popular alternative to soil termiticide applications because bait systems dramatically reduce the amount of pesticide applied in the environment (Su, 1994; Potter et al., 2001a; Su, 2001; Su, 2002). Liquid applications are intended to provide a complete barrier of pesticide between the structure above ground and the termites below. Thus, the use of liquid termiticides may require that hundreds of gallons of dilute pesticide be applied beneath and around a structure, as well as inside, at termite entry points (Potter 1997). Termite bait systems, however, contain relatively small amounts of toxicant (< 1g/bait, Sentricon™, Exterra™, FirstLine™; pesticide label information) confined within a station. Termite foragers feed at the station and then spread the toxicant to other members of their colony via trophallaxis (Ostaff and Gray, 1975; Esenther and Beal, 1979; Jones, 1989, Su and Scheffrahn, 1990a; Stansly et al., 2001). For the majority of baiting systems available, only a small amount of toxicant (e.g. ~1 gram, Sentricon Termite Elimination System; (Su, 1994) is needed to suppress or eliminate a termite colony.

Although subterranean termite baiting systems have the advantage of being more "environmentally friendly" than liquid applications, a persistent concern about termite baiting has been the relatively long time (weeks or months) it takes for termites to locate and feed at the stations (Potter et al., 2001b). In Kentucky, Potter (1997) observed that one infested home had termite activity in more than 50% of the bait stations in as little as 2 weeks, while another infested home in the same neighborhood had no stations "hit" for a year. Likewise, Henderson et al. (1998) found that only 7% of 428 wood monitors were attacked in a 13-month period even though they were placed in very close proximity to actively feeding Formosan termites.

There are several reasons why subterranean termites may take a long time to locate monitors and bait stations in the soil. Puche and Su (2001a) determined that subterranean termites were unable to locate food sources within the soil over distances greater than 2.5 mm. Therefore, termites would be unable to detect bait stations without tunneling almost directly into them. The possibility of termites encountering a bait station while foraging is further reduced by the wide spacing (industry standard is 10' intervals; Potter, 1997) and relatively small size of bait stations (range is ~3-15 cm diameter) in the field.

Currently, there are no practical means of directing or attracting termites into bait stations. However, some of the natural influences on termite foraging behavior could possibly be manipulated to direct their tunneling into a bait station. It has been documented that subterranean termite foraging behavior is frequently influenced by the physical guidelines and food resources they encounter in the soil (King and Spink, 1969; Beard, 1974; Becker, 1972; Rotramel, 1998; Reinhard et al., 1997). Thus, it may be possible to use guidelines to direct termite foraging behavior and lead them into a particular food resource (station).

Several researchers have successfully used laboratory assays as a means to evaluate termite foraging response to obstacles, guidelines, and food resources in the soil (Delaplane and La Fage, 1987; Hedlund and Henderson, 1999; Pitt-Singer and Forschler, 2000; Campora and Grace, 2001). However, none of these studies were designed to direct termite tunneling behavior in an attempt to increase bait discovery. The objectives of this study were to evaluate the influence of physical guidelines on subterranean termite tunneling, and to determine if guidelines could be used to increase termite bait discovery, and consumption.

Materials and Methods

Subterranean termite collection and maintenance. Laboratory studies were conducted to evaluate the potential of physical guidelines to direct termite tunneling and increase bait discovery. *Reticulitermes spp.* were collected from wild populations and maintained at the Dodson Urban Pest Management Laboratory (DUPML) at Virginia Tech in Blacksburg, Virginia. Termites were collected either from in-ground stations or infested wood. The in-ground stations were made of PVC sewer pipe (12 cm diameter) installed on the grounds of the DUPML. The PVC pipes were cut into 42 cm lengths. The lower 30 cm was perforated with 5 mm holes on all sides. The pipe was buried in the soil (30 cm depth) and damp B-corrugated cardboard (Xpedex, Salem, VA) was rolled into cylinders (18 cm x 12cm diameter) and inserted inside the pipe. The station was then closed with a removable PVC cap on the above-ground end. When termites infested the station, the cardboard and termites were removed and placed into a snap top, plastic storage container (15cm x 12cm x 30 cm; Newell Rubbermaid Inc., Freeport, IL). Storage containers contained damp, brown, recycled, single fold paper towels (Prolink™, San Antonio, Texas). Termites in the closed containers were stored in unlighted wooden cabinets and maintained at room temperature. No attempt was made to control the relative humidity in the storage area because of the relatively constant humidity inside the containers (≈ 80% relative humidity). When the paper inside the containers appeared to be dry, 20 ml of water was poured onto the towels to dampen them.

Termites collected in the field were harvested from naturally infested wood gathered on roadsides and in forested areas in the piedmont, coastal plain, and ridge and valley geological provinces of Virginia. Infested wood was transported to the DUPML. Termites were extracted by placing infested wood on damp paper towels with open ends of termite galleries contacting the paper. The wood was allowed to dry naturally. As the wood desiccated, the termites left the wood and entered the damp paper. Once the paper became infested it was removed and placed in a plastic storage container as described above, and fresh paper was placed under the wood to extract additional termites. When termite infestation of the paper towels slowed or ceased, the wood was opened with a hatchet and the remaining termites were extracted.

Laboratory bioassay arenas. Assay arenas consisted of Nunc™ bioassay dishes (23 cm x 23 cm x 2 cm; Nalge Nunc International, Rochester, NY). Four 2 cm discs of brown paper towel (baits) were dampened with distilled water and placed on the bottom of the Nunc™ dish approximately 1cm from the outer wall at the midpoint of each side of the dish (Figure 3.1). Placing the baits away from the outer wall of the dish was intended to discourage discovery of baits by termites tunneling along the outer edges of the arena. One physical guideline was placed in each dish so that it connected two of the four baits. The

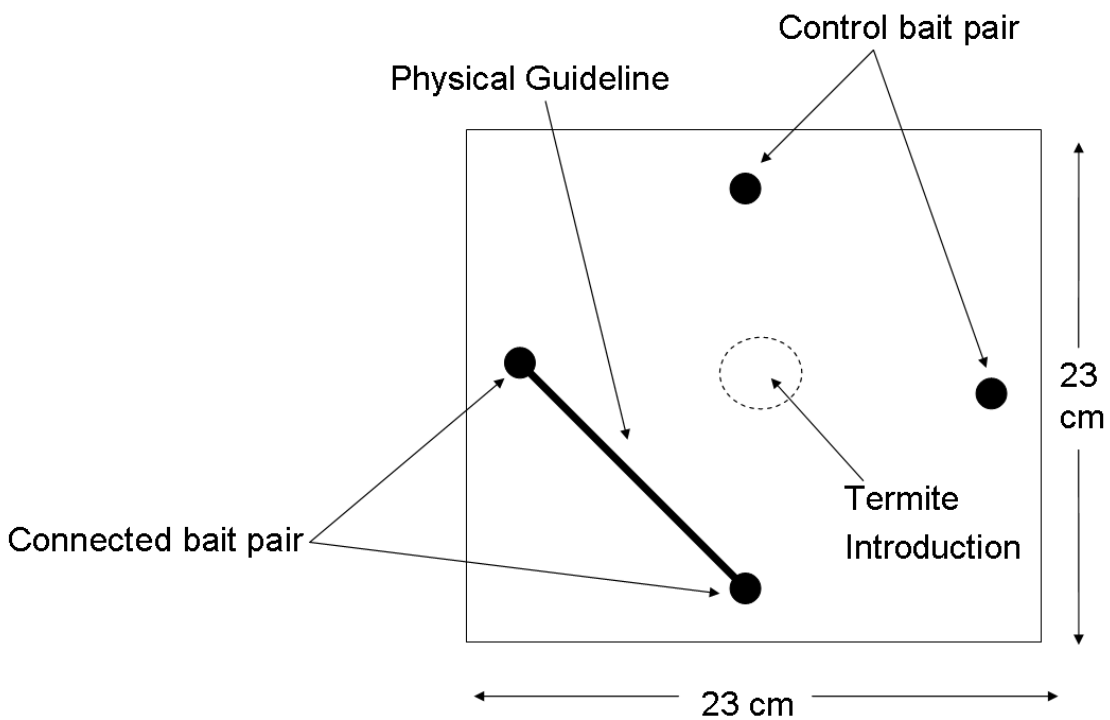


Figure 3.1. Design of the bioassay arena showing the position of bait pairs, the guideline, and the point of introduction for termites.

remaining two baits were left unconnected. Control arenas were prepared as described above, except that no baits were connected by guidelines.

Prior to use, sand (Playsand, Quikcrete™ Corporation, Atlanta, GA) was washed in tap water and dried in an oven for $\geq$ 48 hours at 270° C. The clean dry sand was then divided into 500 g units. Aliquots of sand were stored in plastic storage bags. Distilled water (75 ml; 13% by weight) was added to each bag of sand. The bag was sealed and kneaded by hand to mix sand and water evenly. Damp sand was stored for ~8 hours before being placed in arenas to ensure even moisture distribution. Moistened sand was placed into the assay dishes and spread over the baits and the guideline using a plastic ruler.

Physical guideline materials: wood, and plastics. A variety of materials were tested as physical guidelines (Table 3.1). These materials included white pine (*Pinus strobus* Linnaeus), a wood commonly used for construction in North America, polyvinyl chloride (PVC), a plastic used for plumbing lines and conduit, acrylonitrile butadiene styrene (ABS), a plastic used for corrugated drain lines under structures, high density polyethylene (HDPE), a plastic used for grocery bags, and Trex™, a type of cellulosic plastic known as a wood thermoplastic composite (a hybrid of HDPE and finely ground hardwood sawdust) used as a substitute for wooden landscape timbers and exterior decking. All guideline materials were cut into uniform strips (0.5 cm x 0.5 cm x 12 cm) using a band saw (Sears, Roebuck and Company, Chicago, IL). Each guideline assay and the control assays were replicated fourteen times.

Physical guideline materials assayed: wood thermoplastic composites. Two additional materials (Table 3.1) were tested at a later date for comparison with the pine and Trex™ guidelines. The additional guideline materials were wood thermoplastic composites also used as landscape timbers and exterior decking: Choicedek Classic™ and Fiberon™. Each of these guideline materials and the controls had eight replications.

Table 3.1. Physical guideline materials tested, in the assay of wood and plastic guidelines and the assay of wood thermoplastic composite guidelines, for the ability to direct subterranean termite tunneling, under laboratory conditions.

Guideline Material	Content	Distributor
Wood and Plastics Assay		
pine*	untreated white pine	Heavener's Hardware Blacksburg, VA
ABS	acrylonitrile butadiene styrene	McMaster-Carr Supply Co. New Brunswick, NJ
HDPE	high density polyethelene	McMaster-Carr Supply Co. New Brunswick, NJ
PVC	Polyvinyl chloride	McMaster-Carr Supply Co. New Brunswick, NJ
Trex™*	Hardwood flour (40-60%) Recycled HDPE (40-60%)	Trex Company Winchester, VA
Wood Thermoplastic Composites Assay		
Choicedek Plus™	heartwood cedar fibers (40-60%)	Advanced Environmental Recycling Technologies, Springdale, AZ
Fiberon™	oak & other recycled woods (40-60%)	Unity Forest Products Yuba City, CA

* Materials designated with an asterisk were used in the bioassay comparing wood, and plastic guidelines, and the bioassay comparing wood thermoplastic composite guidelines.

Bioassay design. Prior to testing, populations of termites (50 workers and 1 soldier) were removed from the storage containers with an aspirator and transferred into plastic Petri dishes (4cm, Becton Dickinson Labware, Lincoln Park, NJ). Each dish contained ≈1 cm^2 of recycled brown paper towel from the storage container. The lid was placed on the Petri dish and the termites were allowed to acclimate for 8 h. After the acclimation period, the lid of the Petri dish was removed and the dish was inverted into the center of one of the Nunc™ bioassay arenas. The termites were allowed to forage in the arena *ad libitum* for 72 h.

After the foraging period, the assay arenas were placed on a digital scanner (150 dpi, grayscale) and an electronic image of bait discovery and gallery formation was recorded. Bait discovery and consumption was recorded for each bait that the termites contacted, whether it was connected to a guideline or not. To determine bait consumption, a sheet of clear plastic marked with a 1 mm grid was placed over the image of each discovered bait on the computer monitor. The area consumed was colored in with an erasable felt-tip pen. The number of colored squares on the grid was counted and recorded as a percentage of the total image. The percentage consumption recorded from the image was then used to calculate the actual consumption (mm^2) of the 2 cm diameter bait. Gallery length (cm) along the physical guideline was also measured from the scanned image. Tunnel length was quantified by measuring the tunnel image and then recording it as percentage of the length of the guideline image (both sides of the guideline were included in the calculation because termites could tunnel along both sides). The image tunnel length was then converted to the actual tunnel length by multiplying the percentage times the 12 cm guideline length.

Statistical analysis. Mean tunnel length along each guideline type was compared using Analysis of Variance (ANOVA). Means were separated using Fisher's test of Least Significant Difference (SAS Institute, 1999). Bait discovery and consumption data were analyzed using Chi-Square tests based on a generalized linear model (PROC GENMOD, SAS Institute 1999). For all tests $\underline{P}$ values of ≤ 0.05 indicated significance.

Results

Assays comparing the influence of white pine, Trex™, and plastic guidelines on termite tunneling behavior indicated that tunnel length was significantly influenced by guideline type. The mean length (cm) of tunnels built along the white pine guidelines was significantly greater than the mean length of tunnels built along the ABS, HDPE or PVC guidelines (Figure 3.2; $F = 2.63$, $df = 4$, $\underline{P} = 0.04$). The mean length of tunnels built along the Trex™ guidelines fell between the pine and the plastic guidelines and was not significantly different from either.

Because the results of this first assay indicated that the pine and possibly the wood thermoplastic composite, Trex™ could influence termite tunneling behavior, two additional wood thermoplastic composite (WTC) products,

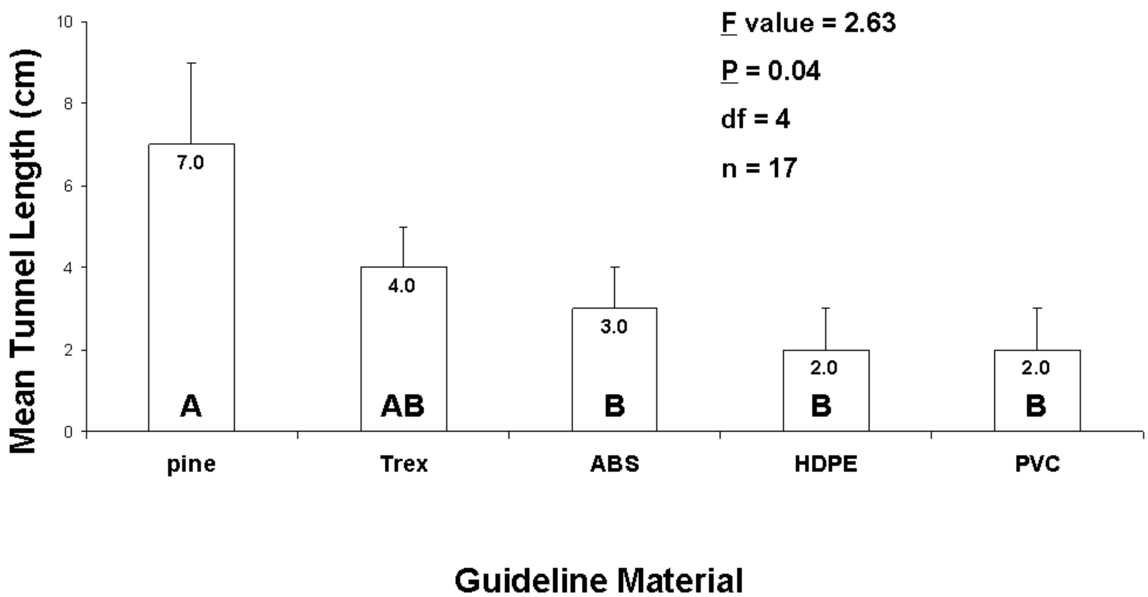


Figure 3.2. Average length (cm) of tunnels built adjacent to plastic (ABS, PVC, HDPE) pine and cellulosic plastic (Trex) guidelines by *Reticulitermes* spp., under laboratory conditions. Lines at the top of the bar represent SEM. Mean tunnel lengths followed by the same letter are not significantly different (P=0.04). One-way Analysis of Variance (SAS Institute, 1999).

Fiberon™ and ChoiceDek™ were evaluated as guideline materials. The results of these subsequent assays indicate that tunnel length along the WTC guidelines was similar to that of the white pine and Trex™ guidelines. Although the mean tunnel length appeared to be greater along the three WTC products than the white pine (Figure 3.3), the differences were not significant ($F = 1.17$, $df = 3$, $\underline{P} = 0.34$).

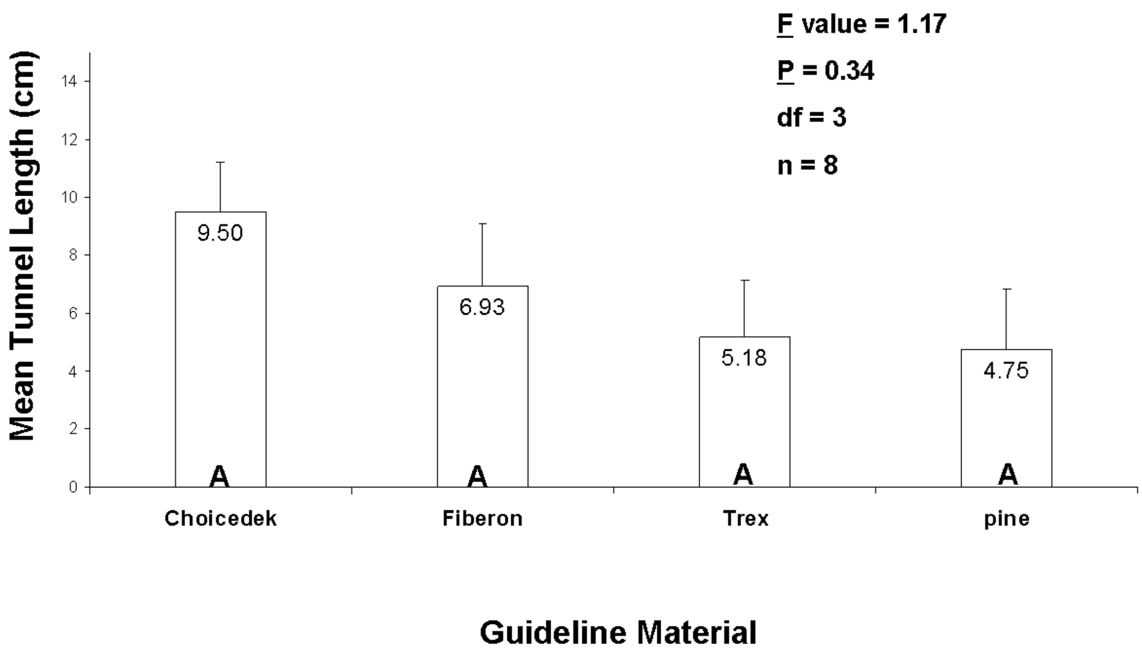


Figure 3.3. Average length (cm) of tunnels, built by *Reticulitermes* spp, adjacent to white pine and wood thermoplastic composite guidelines (Choicedek, Fiberon, Trex), under laboratory conditions. Lines at the top of the bar indicate SEM. Mean tunnel lengths followed by the same letter are not significantly different. One-way Analysis of Variance (SAS Institute, 1999).

Figure 3.4 illustrates the percentage of arenas in which one or both baits, connected by a guideline (or not connected for the controls) were discovered by foraging termites. The presence or absence of a guideline did not affect termite discovery of just one bait ($\chi^2 = 0.11$ df = 2, $\underline{P} = 0.74$). Likewise, the guideline material had no influence on termite discovery of one bait associated with that guideline ($\chi^2 = 6.82$, df = 4, $\underline{P} = 0.15$). However, the discovery of both baits (bait

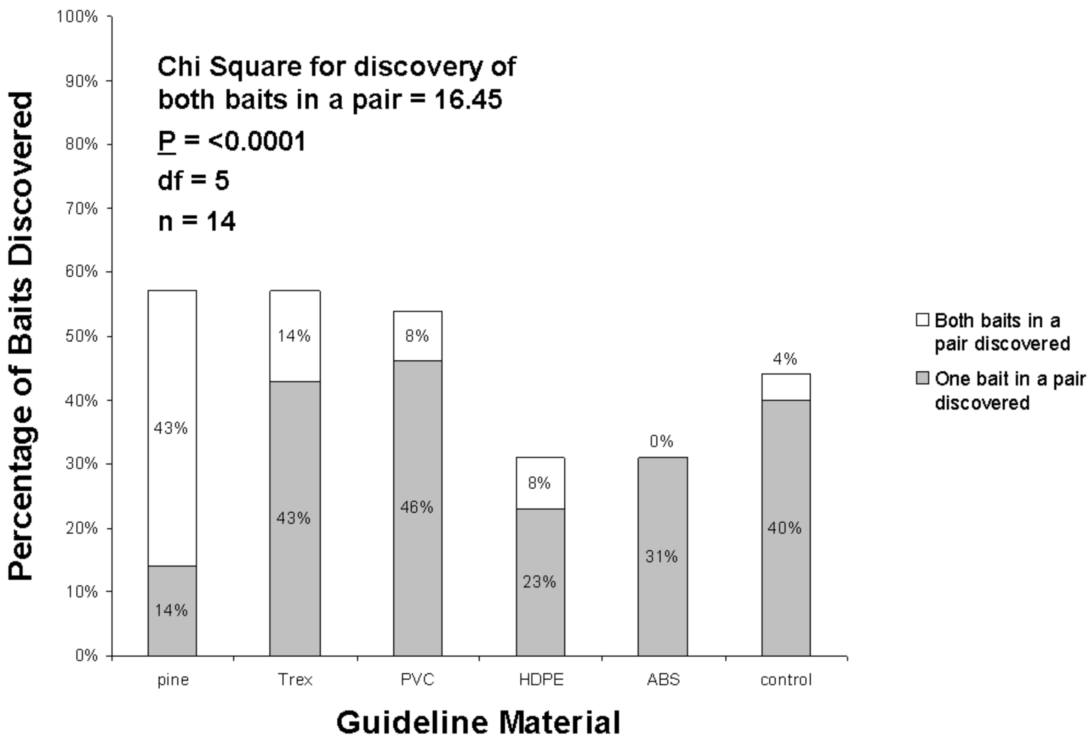


Figure 3.4. Percentage of baits, connected by plastic (ABS, PVC, HDPE) pine and cellulosic (Trex) physical guidelines, discovered by foraging *Reticulitermes* workers in a laboratory bioassay. (P = <0.0001) χ^2 Test; PROC GENMOD, SAS Institute, 1999).

pairs) was influenced by guideline type. The percentage of bait pairs connected by white pine guidelines that were discovered (43%) was significantly greater than the pairs of baits connected by plastic guidelines and the unconnected controls (χ^2 = 11.99, df = 5, P = 0.0005). There was no significant difference in the percentage of bait pairs discovered for baits connected by any of the three plastics tested (χ^2 = 1.67, df = 2, P = 0.43). The percentage of pairs of baits connected by Trex™ guidelines that were attacked did not differ significantly from the percentage connected by pine ($\chi^2 = 2.90$, df = 1, $\underline{P} = 0.09$), or plastic guidelines ($\chi^2 = 2.09$, df = 3, $\underline{P} = 0.15$).

When bait discovery was compared between the white pine and wood thermoplastic composite guidelines, it was again determined that the discovery of individual baits connected by guidelines was not significantly different from the percentage of unconnected baits discovered ($\chi^2 = 3.34$, df = 1, $\underline{P} = 0.07$). However, the probability of both baits in a pair being discovered was significantly greater for those baits connected by a guideline ($\chi^2 = 21.57$, df = 4, $\underline{P} < 0.0001$). Interestingly, the discovery of both baits in a connected pair was not significantly different for any of the guideline materials tested, indicating that the wood thermoplastic composites were just as likely to influence termite discovery of two baits as the white pine guideline ($\chi^2 = 0.6$, df = 3, $\underline{P} = 0.8$; Figure 3.5).

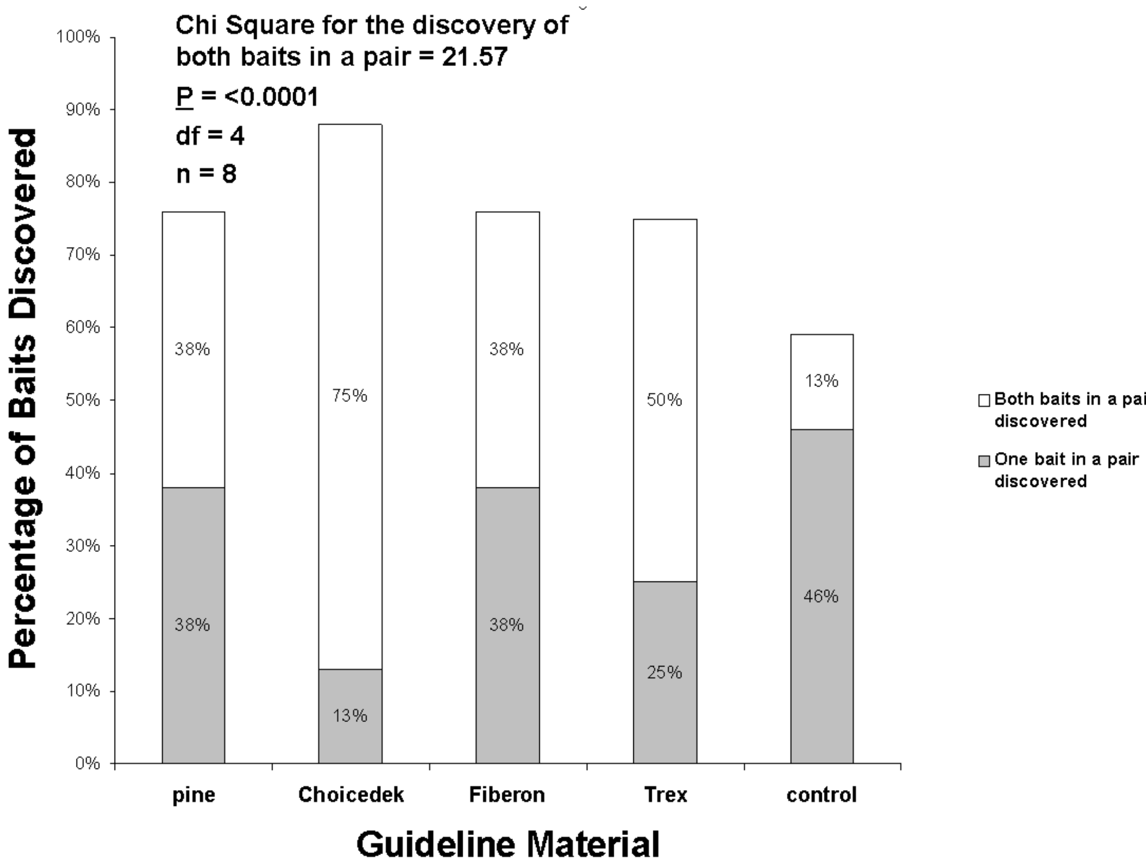


Figure 3.5. Percentage of baits, connected by white pine and wood thermoplastic composite (ChoiceDek™, Fiberon™, Trex™) physical guidelines, discovered by foraging Reticulitermes spp. workers in a laboratory bioassay. Subterranean termites were significantly more likely to discover both baits in a connected by wooden or cellulosic guidelines than both unconnected control baits**.** (P = <0.0001; PROC GENMOD, SAS INSTITUTE, 1999).

The mean consumption of baits connected by the wood, Trex™ and plastic guidelines is recorded in Table 3.2. Mean consumption (mm^2) of two baits connected by a physical guidelines (10.3 ± 2.1 mm^2) did not differ significantly from consumption of pairs of unconnected baits (11.5 ± 2.7 mm^2; $\chi^2 = 0.07$, df=1,

Table 3.2. Mean proportion (mm2) of total bait consumed, within a bioassay arena, for pairs of connected baits associated with physical guidelines and control baits (no guideline).

Guideline Material	Mean $\pm$ SEM (%)	χ^2	P
Trex™	63.5 $\pm$ 14.4	7.39	0.59
PVC	58.6 $\pm$ 13.5		
pine	57.4 $\pm$ 13.3		
control	48.8 $\pm$ 6.0		
ABS	33.6 $\pm$ 16.2		
HDPE	33.4 $\pm$ 15.6		

χ^2 test (PROC GENMOD, SAS Institute, 1999).

P = 0.79). Although the likelihood of two baits being discovered was greater in the presence of the wood guideline, the presence of physical guidelines in general did not influence bait consumption nor was consumption influenced by guideline type.

Discussion

Overall, the results of this study indicated that subterranean termites did not respond to all guideline materials in the same way. Figure 3.6 illustrates two of the typical responses observed when the termites encountered a plastic guideline. The guideline was not explored even if the termites contacted it multiple times. Instead the guideline was treated as an obstacle to tunnel around

(Figure 3.6a) or under (Figure 3.6b). Termite response to the white pine was obviously different (Figure 3.7). In almost every replication, except those where the termites never encountered the guideline at all [4 replications (18%) of 22 total replications], the termites would tunnel up one side, discover the first bait and then continue tunneling down the other side of the guideline, frequently discovering the second bait. Termites responded to the wood thermoplastics as they did the white pine guidelines, frequently tunneling along both sides of the guidelines and discovering both baits.

There are a number of reasons why termites might tunnel along wood guidelines more readily than plastic. The most obvious is that wood is a food source frequently encountered in the termites' natural environment. Several studies have shown that termites tunnel extensively along objects that they recognize as food (Williams, 1934, Goldberg, 1973a, Hedlund and Henderson 1999, Cornelius and Osbrink 2001, Puche and Su 2001b). Reinhard et al. (1997) suggested that subterranean termites use exploratory tunneling to evaluate the size and quality of a food source. Additional studies have determined that once termites discover a food resource in the soil, this food resource becomes a new center of foraging activity leading to the discovery of additional resources in the same area (King and Spink 1969, Campora and Grace 2001, Puche and Su 2001a). The termite response to the wood guidelines in our assay supported these studies. Once the termites had encountered the wood guidelines, their

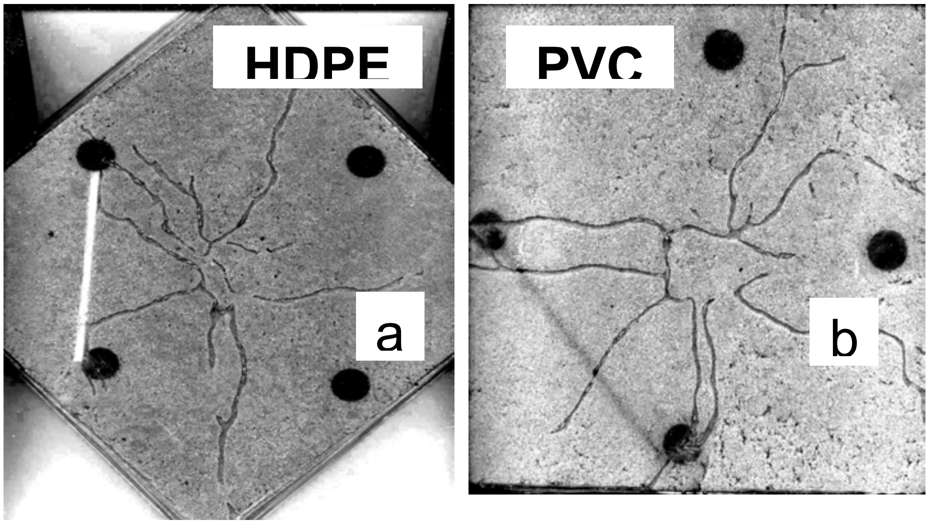


Figure 3.6. Subterranean termites tunneling in Nunc bioassay arenas containing plastic guidelines. a) Termites did not explore plastic guidelines. b) Termites tunnel under a plastic guideline.

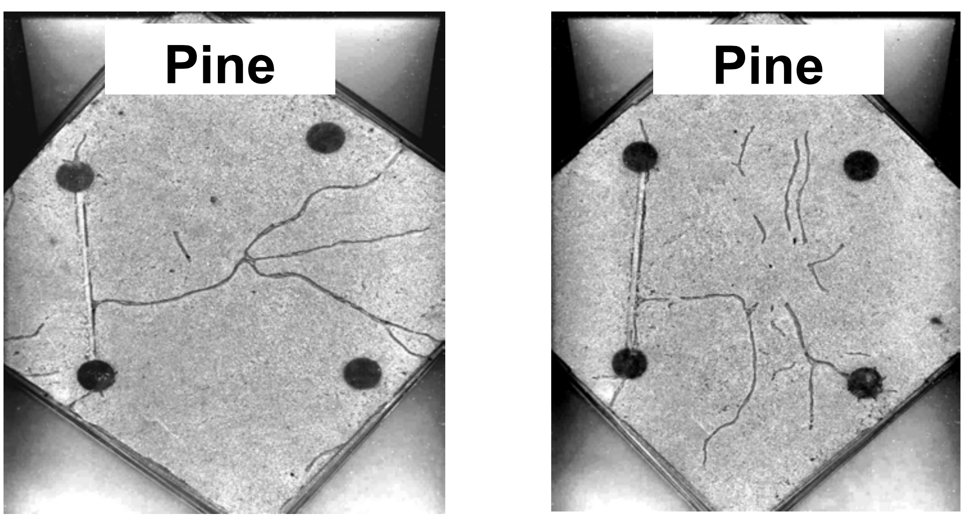


Figure 3.7. Termites tunneling in Nunc bioassay arenas containing white pine guidelines. *Reticulitermes* spp. tunneled adjacent to the entire length of the guideline, exploring both sides.

exploratory tunneling frequently led them to discover additional baits associated with those guidelines.

Field studies have documented that subterranean termites will also tunnel along living roots on which they are not feeding (King and Spink 1969). Jander and Daumer (1974) identified similar behavior in termites foraging above ground in Australia and termed it "guideline following". The use of wood guidelines on which they do not feed, may provide termites with an advantage of following the path of least resistance to a more palatable food resource, such as fallen wood, associated with the living roots (Jander and Daumer 1974). This "guideline following" behavior associated with less palatable wood may help to explain the termites' response to wood thermoplastic composites in our assays. Although we did not measure consumption of any of the guidelines tested, we observed termite etching on some of the white pine guidelines. We did not observe any etching on the wood thermoplastic composites, which are designed to be termite resistant (Forest Products Society 2001). However, we speculate that the wood fibers in the composites may have provided termites with some of the olfactory and gustatory cues that they respond to in natural wood food sources, and that these cues may have been enough to evoke the "guideline following" response described by Jander and Daumer (1974). This "guideline following" subsequently led to the discovery of baits associated with the WTC guidelines.

Several researchers have used the terms "guideline following" to describe termite tunneling through cracks in mortar, along plumbing pipes and other architectural features of construction (Thorne and Forschler 1998). The fact that these guidelines do not necessarily contain wood suggested that many types of materials might serve as foraging guidelines by providing a path of least resistance through the soil. Pitt-Singer and Forschler (2000) found in laboratory assays that *Reticulitermes* species would follow pre-existing tunnels and guidelines made of wire through soil. Goldberg (1973b) also found that termites would use the side of their Plexiglas container as a physical guideline in laboratory assays, until an obstacle was put in their path.

In developing our bioassay, we had anticipated that plastic construction materials would make ideal guidelines for several reasons. The plastic materials were durable and would not deteriorate in the soil. In addition, all of the currently available bait stations are made of plastic so stations and guidelines could be manufactured together. Finally, and perhaps the most compelling reason was that two commercial bait systems had already incorporated plastic guideline elements in their design. The FirstLine System (FMC Corp. 1999) has guidelines molded into the design of their SmartDisk, the grade-level cap of the in-ground station. The guidelines in the cap of the station are intended to “channel” termites into the station’s core once the station has been discovered (FMC Corp. 1999). The Termatrol™ monitoring/baiting system also had manufactured guidelines that could be purchased and attached to the regular termite monitoring stations. The Termatrol™ guidelines were composed of a molded plastic sheath perforated with holes that covered a removable piece of wood. Up to four of these guidelines could be attached to the station and the station installed in the ground with the guidelines radiating out in all four directions. However, our studies indicate that in spite of the potential benefits of using plastic materials, plastics made less than ideal guidelines for termites. Termite tunneling was minimal along the plastic guidelines and therefore, fewer connected baits were discovered. In addition, it should be noted that in early replications (November 1, 2001), when the plastic guidelines were freshly cut, the termites actually appeared to avoid contact with PVC guidelines and, in many cases, failed to build any tunnels in the area of the Nunc dish containing the PVC guideline. The foraging termites produced uncharacteristic, lopsided tunnel configurations instead of the characteristic star pattern. This response seemed to indicate that the *Reticulitermes* spp. actually found the PVC guidelines repellant. In later replications, the termites produced normally distributed gallery configurations in the dishes containing PVC guidelines. It seems possible that freshly cut PVC may have produced repellant volatiles, which eventually dissipated. In any case, the termites' behavior in dishes containing PVC guidelines raises serious questions about the use of plastics to construct guidelines and even bait stations. Coincidentally, Termatrol™ no longer offers guidelines as part of their product line (Termatrol™ 2003), and we could find no literature evaluating their efficacy.

Although our results indicated that wood and wood thermoplastic composites made superior guidelines, bait consumption was not increased by their presence. In all of the bioassay arenas, with or without guidelines, the termites found at least one bait during the test period. In arenas that contained no guidelines or plastic guidelines, the termites would concentrate their consumption on the first bait discovered. Only after that first discovery was near depletion would they begin foraging again. Likewise, in arenas where baits were connected by wood and wood thermoplastic composites, termites would tunnel along the guideline, discover the first bait, and feed. However, termite tunneling would still continue along the wood or WTC guideline until the second bait was discovered. Termites would feed on the second bait, but the majority of consumption was concentrated on the first bait. Because a group of termites can only consume a certain amount of bait in a given time, the collective consumption between multiple baits was not greater than the consumption of one bait alone.

We recognize that the wood, and possibly the Trex™ (only WTC tested where we measured consumption), guidelines were competing with the baits for the termite consumption in these bioassays. Although the presence of the wood guidelines did not reduce bait consumption significantly, we would expect that their influence on consumption would be greater had the assays been run for a longer period of time.

Summary. Guidelines made of wood or wood thermoplastic composites were able to direct the tunneling of subterranean termites and subsequently increased the number of baits discovered. Termites did not explore the plastic guidelines therefore; the discovery of multiple baits was less frequent. The amount of bait consumed by the termites was not increased by the presence of any type of guideline. Even though the wood guidelines aided the termites in the discovery of multiple baits, termites would not consume more of these multiple baits than they would of a single bait.

In this study, we have presented data suggesting that guidelines made of WTC have the potential to enhance termite discovery of bait stations. WTCs are designed to be resistant to wood decay fungi and do not degrade readily in the soil. Also, because WTCs are designed to be resistant to termite attack (Forest Products Society 2001), they would not readily compete with bait stations as a food resource, which would be a significant problem with guidelines made of wood.

It is interesting to note that all currently available bait stations are made of plastic. As we have demonstrated, plastics, though durable, may not be the most attractive material to subterranean termites. It is possible that molding stations out of wood thermoplastic composites would be a better option. Because the termites explore the WTCs in much the same way as they do wood (a food resource), termites might explore WTC stations more thoroughly than plastic stations. Termites might also be more likely to discover additional stations located in the same areas because the bait station itself would represent a potential center of foraging activity (food resource; Campora and Grace 2001) not just the bait inside. Another advantage of the WTC materials is that the ratios of wood fibers to HDPE could be formulated for maximum attractiveness to termites while maintaining the material's durability. The potential of these novel wood thermoplastic composites is certainly worthy of further investigation. The ability of WTCs to influence termite discovery of wood monitors in the field will be presented in the following study.

CHAPTER IV: SUBTERRANEAN RESPONSE TO PHYSICAL GUIDELINES IN THE SOIL IN VIRGINIA FIELD SITES

Introduction

Subterranean termites (Isoptera: Rhinotermitidae) are the most ubiquitous structural pests in the United States (Pawson and Gold, 1996) causing billions of dollars in damage each year (Su and Scheffrahn, 1990a). Because of the subterranean termite's pest status, control methods have been the subject of intense scientific investigation throughout much of the twentieth century (Kofoid, 1934b; Hatfield, 1944; Esenther and Coppel, 1964; Williams, 1977; Su et al, 1987; Nutting and Jones, 1990; Grace, 1991; Kard, 1999; Henderson, 2001). As a result of these studies, a number of products have been developed to eradicate or prevent subterranean termite infestations (Su and Scheffrahn, 1990a; Su and Scheffrahn, 1998; Ring et al., 2001; Verkirk and Bravery, 2001; Su, 2002).

Subterranean termite control products employ one of three strategies for managing subterranean termites: installation of physical or chemical barriers to prevent termites from attacking structural wood, impregnation of wood with a preservative, or destruction of the termite nest (French, 1991). In North America, the most common treatment for subterranean termites consists of the application of liquid termiticide to the soil between the structure and termite colony (Ostaff and Gray, 1975; Su and Scheffrahn, 1990a; Koehler, et al., 2000; Su, 2001; Su et al., 2001).

There are several limitations to controlling subterranean termites with liquid termiticides. First, it is difficult to create a continuous horizontal barrier beneath an infested structure by applying liquid termiticide (Su and Scheffrahn, 1990a; Koehler et al., 2000). Also, conventional applications rely on the introduction of large volumes of highly residual insecticides into the soil around the structure (Esenther and Beal, 1979; Su and Scheffrahn, 1990a). Many people are concerned about the long-term effects of liquid termiticides in their immediate environment. (Ostaff and Gray, 1975; Lewis, 1980; Jones, 1989; Su and Scheffrahn, 1990a; French, 1991; Wood and Pierce, 1991; Peyton et al., 1995; Sim et al., 1998; Pawson and Gold, 1996; Potter et al., 2001; Verkerk and Bravery, 2001). Finally, liquid termiticides are disruptive to the structure. Liquid applications to mitigate existing infestations usually require drilling through porches, foundation block, veneers and patios to deliver the termiticide to the proper location (Koehler et al., 2000; Potter and Hillery, 2001).

One alternative to liquid termiticide has been the use of termite baiting systems, which are environmentally friendly and not disruptive to buildings (Ostaff and Gray, 1975; Esenther and Beal, 1979; Su and Scheffrahn, 1990a; French, 1991; Koehler et al., 2000; Potter et al., 2001; Verkerk and Bravery, 2001). Baits work by suppressing or eliminating termite colonies. Foraging termites locate and consume the bait. They then transport it back to the nest where it is distributed to other colony members by social interaction i.e. grooming and trophallaxis (Ostaff and Gray, 1975; Esenther and Beal, 1979; Jones, 1989; Su and Scheffrahn, 1990a; Stansly et al., 2001).

While subterranean termite baiting systems have many advantages over liquid termiticides, however, because none of the subterranean termite baits currently marketed contains an attractant, at present, it is not possible to direct termite foraging activity into a bait station (French, 1991; Pawson and Gold, 1996; Potter et al., 2001). Termite discovery of and recruitment to the bait is unpredictable. Months or even years may pass before a termite baiting system is discovered by foraging termites (French, 1991; Ring et al., 2001; Verkirk and Bravery, 2001).

Our current understanding of termite foraging strategies suggests one way to decrease bait discovery time. It has been documented that subterranean termites frequently follow physical guidelines in the soil (King and Spink, 1969; Pitts-Singer and Forschler, 2000; Campora and Grace, 2001; Grace and Su, 2001). By placing guidelines between in-ground bait stations, it might be possible to increase bait discovery by directing foraging termites into the stations.

In a series of bioassays described in the previous chapter, it was documented that, under laboratory conditions, foraging subterranean termites were more likely to follow physical guidelines containing cellulose than guidelines with no cellulosic component. It was determined also that Trex™ and other wood thermoplastic composites were as effective at directing subterranean termite foraging activity as white pine. Thus we chose to test guidelines composed of white pine and Trex™, a wood thermoplastic composite, in the field.

Although we had demonstrated that plastic guidelines were significantly less effective than materials containing cellulose at directing *Reticulitermes* spp. movement through the substrate we chose to include guidelines composed of HDPE as a control. In addition, and perhaps the most compelling reason we included HDPE in the field study was that two commercial bait systems had already incorporated plastic guideline elements in their design. The FirstLine™ System (FMC Corp. 1999) has guidelines molded into the design of their SmartDisk™, the grade-level cap of the in-ground station. The guidelines in the cap of the station are intended to "channel" termites into the station's core once the station has been discovered (FMC Corp. 1999). The Termatrol™ monitoring/baiting system also had manufactured guidelines that could be purchased and attached to the regular termite monitoring stations. The Termatrol™ guidelines were composed of a molded plastic sheath perforated with holes that covered a removable piece of wood. Up to four of these guidelines could be attached to the station and the station installed in the ground with the guidelines radiating out in all four directions. However, our studies indicate that in spite of the potential benefits of using plastic materials, plastics made less than ideal guidelines for termites. Termite tunneling was minimal along the plastic guidelines and therefore, fewer connected baits were discovered. Coincidentally, Termatrol™ no longer offers guidelines as part of their product line (Termatrol™ 2003), and we could find no literature evaluating their efficacy.

The laboratory assays determined how different guideline materials might influence termite guideline-following behavior in isolation from distracting environmental stimuli. However, in the natural environment there are many variables that may affect termite response to physical guidelines. The most obvious is termite population pressure. It is well known that subterranean termites are more prevalent, more active, and more voracious in some locations than in others depending on the environmental conditions (Gentry, 1982; Collins, 1991). In order to fully determine the influence of guideline materials on termite foraging behavior in a natural environment, we needed to supplement the laboratory bioassays with field studies conducted in different types of locations.

It has been demonstrated in the laboratory that subterranean termite tunneling can be directed by physical guidelines buried in the substrate (Pitts-Singer and Forschler, 2000; Campora and Grace, 2001). Researchers evaluating termite colonies in the field have also made qualitative observations that termites follow physical guidelines in their natural environment (Kofoid, 1934a; King and Spink, 1969; French 1991). The purpose of this study was to determine the ability of different physical guideline materials to direct subterranean termite foraging behavior in three field environments.

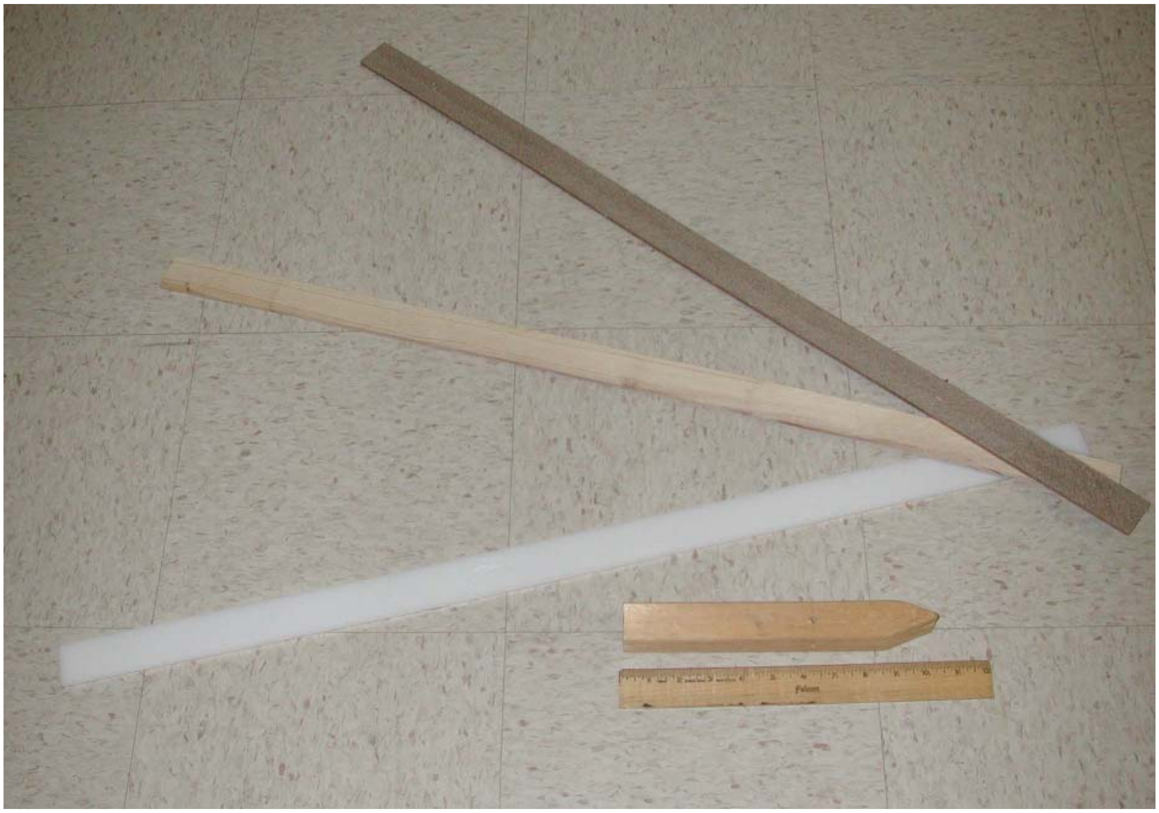


Figure 4.1. Samples of the physical guidelines and wooden bait stake used in the field bioassay of the ability of physical guideline to direct foraging *Reticulitermes* spp. toward bait. Shown with a ruler to establish scale.

Materials and Methods

Field assay of physical guidelines. The guideline materials tested were Trex™, white pine, and high-density polyethylene (HDPE). In addition, a physical gap in the soil was evaluated as a guideline. The three guideline materials were cut into uniform strips, (Fig. 4.1; 100 cm long; 4 cm deep). The gap (100 cm x 4 cm x 1 cm) was created using a flat edged spade. The end of the spade was inserted into the soil in order to sever roots and displace stones. The handle of the spade was toggled back and forth perpendicular to the blade to widen the gap (0.5 cm). The gap was cut at the time of field plot installation and was not renewed during the course of the study.

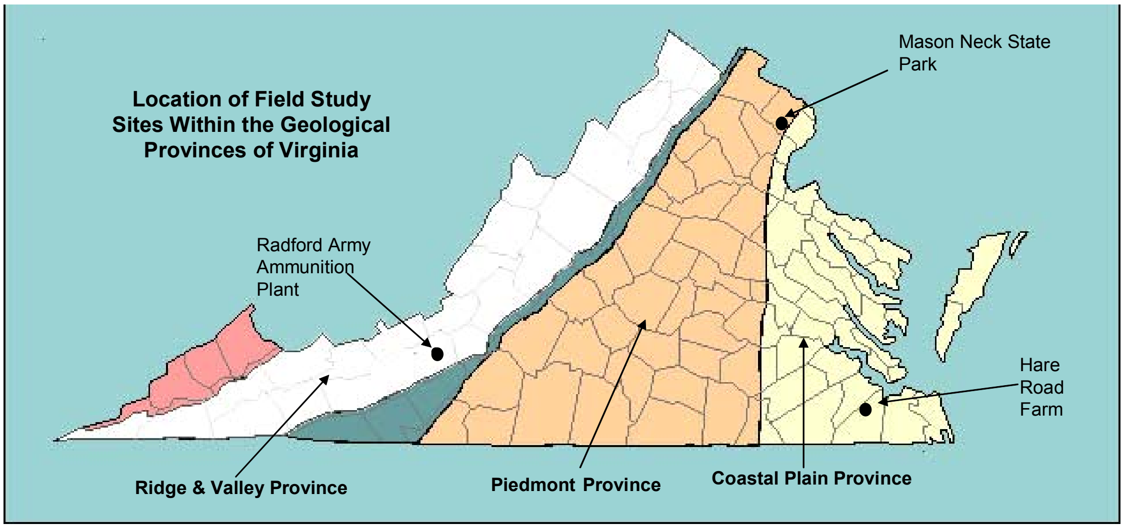


Figure 4.2. Map of field sites: Radford Army Ammunition Plant (RAAP), Montgomery County; Mason Neck State Park (MNSP), Fairfax County; Hare Road Farm (HRF); Suffolk County.

Study sites. Three field sites were established in Virginia for testing physical guidelines (Fig. 4.2; Table 4.1). Sites were selected because they contained abundant evidence of subterranean termite activity (living termites and damaged wood). One field site was located in each of the three largest geological provinces of Virginia: Coastal Plain (Hare Road Farm, agricultural land bordering on a mixed forest; Fig. 4.3), Piedmont (Mason Neck State Park, a wetland area consisting mainly of climax deciduous forest; Fig. 4.4), and Ridge and Valley (Radford Army Ammunition Plant, a 15-year old stand of commercially planted white pine; Fig. 4.5).

Site	County	Altitude (m)	Latitude	Longitude	Annual * Precip. (cm)	Soil Series	Mean * Summer High Temp. (C)	Mean * Winter Low Temp. (C)
Hare Road Farm (H)	Suffolk	72	36°38'48"N	76°32'07"W	117	Woodstown	30.2	-0.5
Mason Neck State Park (M)	Fairfax	10	38°38'5"N	77°10'55"W	98	Emporia Euchee	29.7	-2.1
Radford Army Ammunition Plant (R)	Montgomery	634	37°11'48"N	80°21'02"W	103	Unison	27.3	-5.4

Table 4.1. Geological and climatic characteristics of three field sites established in Virginia to test the ability of physical guidelines to guide *Reticulitermes* spp. foragers to inground baits.

* Data provided by SE Regional Climate Center, 2221 Devine St., Columbia, SC 29205;

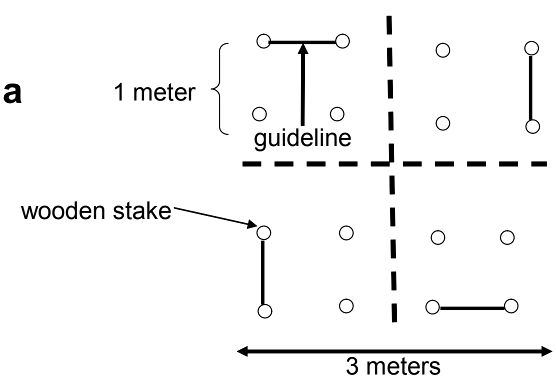
Figure 4.3. The soil surface at Hare Road Farm was free from mulch.

Figure 4.4. A thin layer of damp leaf mulch covered the soil surface at Mason Neck State Park.

Figure 4.5. The soil surface at the Radford Army Ammunition Plant was covered with a thick layer of pine straw.

Plot design. Five replicates (plots) for each guideline material were established at each field site. Each plot was located within 4 meters of an active termite infestation or evidence of significant termite damage. Each plot consisted of sixteen stakes positioned as shown in Fig. 4.6 a (a total of 80 stakes / treatment / field site). Plots were located at least 4 m apart and within 8 m of an active termite infestation. Each plot contained four subplots composed of four stakes each. Each subplot of four stakes contained two stakes connected with a guideline and two unconnected stakes.

a



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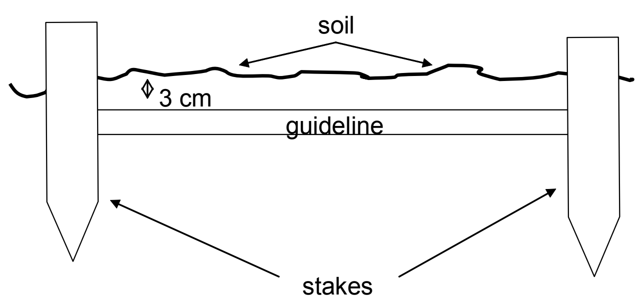

Figure 4.6. a) The position of wooden stakes and guidelines in field plots. Each plot contains only one guideline material (top). **b)** Position of physical guidelines relative to wooden stakes and soil surface (bottom).

Guidelines were buried or cut approximately 3 cm below the surface of the soil and were positioned to connect two adjacent stakes (Fig. 4.6 b). There were four possible positions for the guideline within a set of four stakes and these are illustrated in Fig. 4.6 a. Each subplot of four stakes within one plot had the guideline in a different position. The position of the guidelines within a plot was randomly determined. Each plot contained only one guideline material.

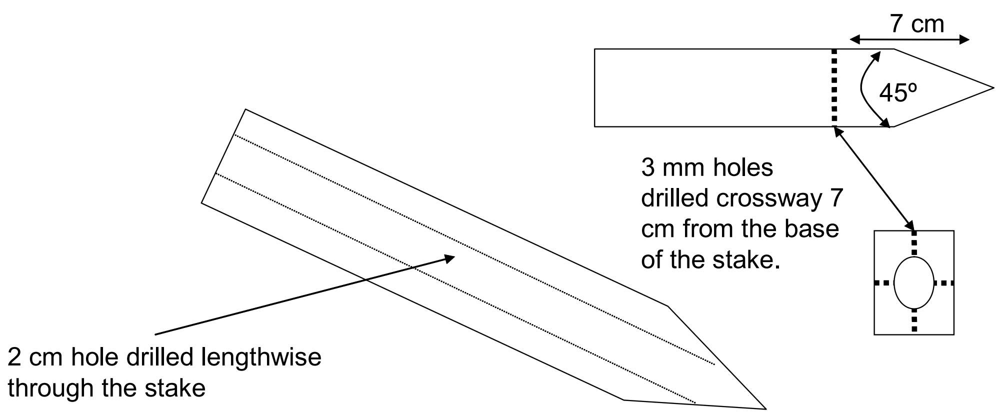

Figure 4.7. Diagram of the wooden stakes used in field plots.

The wooden stakes used in this study (Figs. 4.1 and 4.7) were similar to those described by Ewart et al. (1992). Stakes were constructed of untreated white pine (Heavener's Hardware, Blacksburg, VA), and were cut to a uniform size (22 cm x 5 cm x 5 cm) with a 45° wedge cut on one end. Each stake had a hole drilled lengthwise down the center using a ship's auger drill bit (1 1/2"; Makita USA Inc, LaMirada, CA). Each stake was also perforated crossways by two perpendicular holes located 7 cm above the pointed base using a ¼" drill bit.

After installation in the field, the top of each stake was plugged with a rubber stopper (Prince Rubber and Plastics Co. Inc., Buffalo, NY) that could be removed to permit inspection of the stake's interior.

Data collection. Wooden stakes were examined for subterranean termite infestation once a month from June of 2001 to October of 2002 at each of the three study sites. No inspection was performed during the winter months of 2001 because termite activity is minimal during cold weather. Stakes were inspected by removing the stopper and observing the interior of the stake for termite activity. If live termites were seen, the stake was considered to be infested. When a mud plug was found in the stake, the mud was perforated with a blade. The stake was considered to be infested when live termites were found below the mud plug. If the mud plug was found to be solid and damp at a depth of 2 cm, the stake was worked loose from the ground and inspected for infestation by termites. After inspection, the stake was returned to the same position in the plot. At the end of October 2002 (16 months), all stakes at each site were removed and destructively examined for subterranean termite infestation.

Statistical analysis. The total number of stakes attacked at each test site over the test period was quantified, and the mean number of stakes attacked per plot was compared to determine termite pressure at each location. The proportion of connected and unconnected stakes attacked within the Hare Road Farm and Mason Neck State Park test sites was compared by month using χ^2 analysis based on the generalized models (PROC GENMOD, SAS Institute, 1999). $\underline{P}$ values of ≤ 0.10 indicated significance. The percentage of bait stakes attacked, grouped by type of guideline connecting them, was compared using χ^2 analysis (PROC GENMOD, SAS Institute, 1999). P values of $\leq$ 0.10 indicated significance. We chose to use a less stringent measure of significance than the usual 0.05 for this bioassay, because the field environment was complex and includes numerous influences outside of the basic experimental model that might dilute the effect of the guidelines.

Results

None of the four guidelines tested (white pine, HDPE, Trex™, or gap in the soil) was found to significantly influence termite attack of the wooden stakes Mason Neck State Park (MNSP) or Hare Road Farm (HRF) (χ^2 = 0.36, df = 3, P=0.95; Table 4.2). However, subterranean termite pressure, as indicated by the percentage of stakes attacked over the 16-month test period, varied considerably between the three field sites (Figure 4.8). Because of the sparse data collected at the Radford Army Ammunition Plant it was decided not to further analyze the data from RAAP.

Table 4.2. Comparison of the mean ± SEM of connected stakes attacked by *Reticulitermes* spp. at field sites by guideline type.

Location	Guideline Material	Mean ± SEM Attacked	χ^2	df	P
Hare Road Farm					
	pine	7.2 ± 0.5	0.25	3	0.97
	Trex™	5.8 ± 0.5			
	HDPE	5.2 ± 0.7			
	gap in the soil	7.0 ± 0.5			
Mason Neck State Park					
	pine	4.2 ± 1.4	0.52	3	0.91
	Trex™	4.0 ± 0.9			
	HDPE	3.0 ± 0.84			
	gap in the soil	5.6 ± 0.7			

χ^2Test (PROC GENMOD; SAS Institute, 2003.)

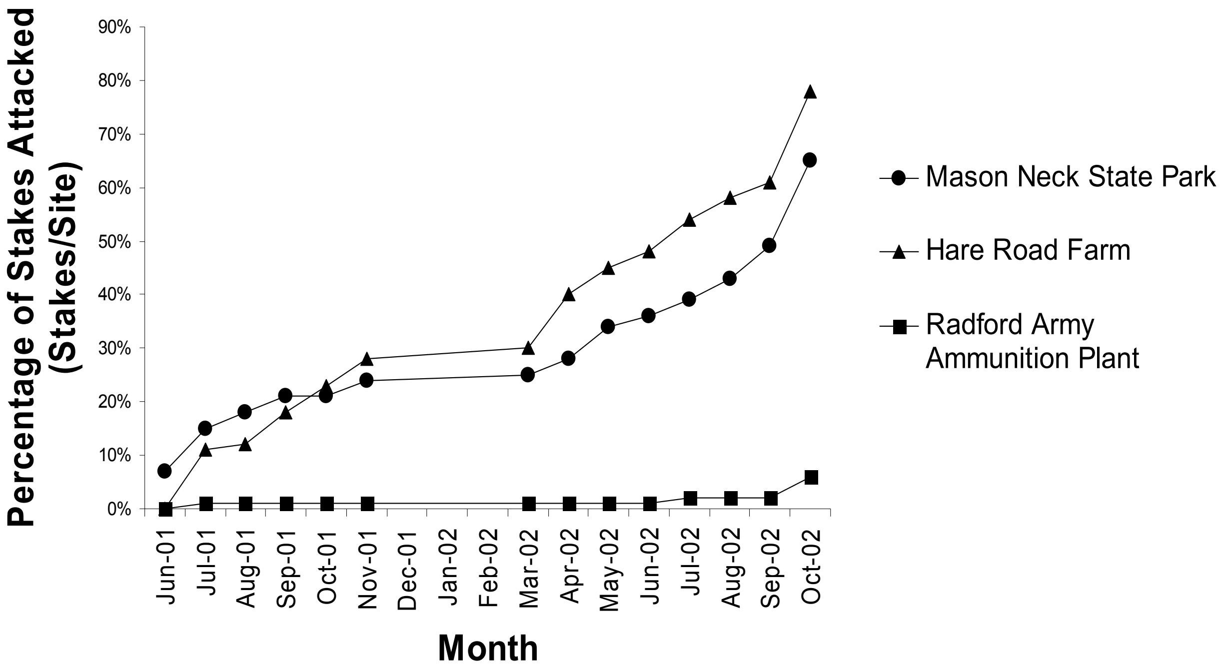

Figure 4.8. The percentage of stakes attacked by termites at the three field sites from June 2001 to October 2002.

Subterranean termite pressure was found to be greatest at Hare Road Farm, where 80% of all stakes deployed were attacked, during the 16-month test period. The mean number of stakes attacked per plot (12.8 $\pm$ 0.7) was significantly greater than the number attacked at Mason Neck State Park (Table 4.3; 10.5 + 0.8; χ^2 = 114.8, df = 2, $\underline{P}$ < 0.001). Termite pressure was extremely low at the Radford Army Ammunition Plan with an average of less than one stake attacked per plot.

Table 4.3. Percent of stakes attacked by *Reticulitermes* spp. at field sites from June 2001 to October 2002[1].

Site	Percent of All Stakes Attacked (n = 320)	Mean $\pm$ SEM Discovered Per Plot (n = 16)[2]	χ^2	df	P
Hare Road Farm	80%	12.8 $\pm$ 0.7 a	114.78	2	<0.0001
Mason Neck State Park	65%	10.5 $\pm$ 0.8 b			
Radford Army Ammunition Plant	6%	1.0 $\pm$ 0.3 c			

[1] χ^2 Test (PROC GENMOD; SAS Institute, 2003.)

[2] Locations with different letter designations are significantly different.

At Hare Road Farm, the proportion of stakes connected by a guideline that were attacked by termites was significantly greater than the proportion of freestanding stakes that were attacked from October 2001 till September 2002 (Table 4.9; χ^2 = 0.05, df = 1, P= 0.82). However, when the stakes were destructively examined in October 2002, the proportion of the total number of stakes attacked by termites was not significantly different for baits connected by a guideline and freestanding baits.

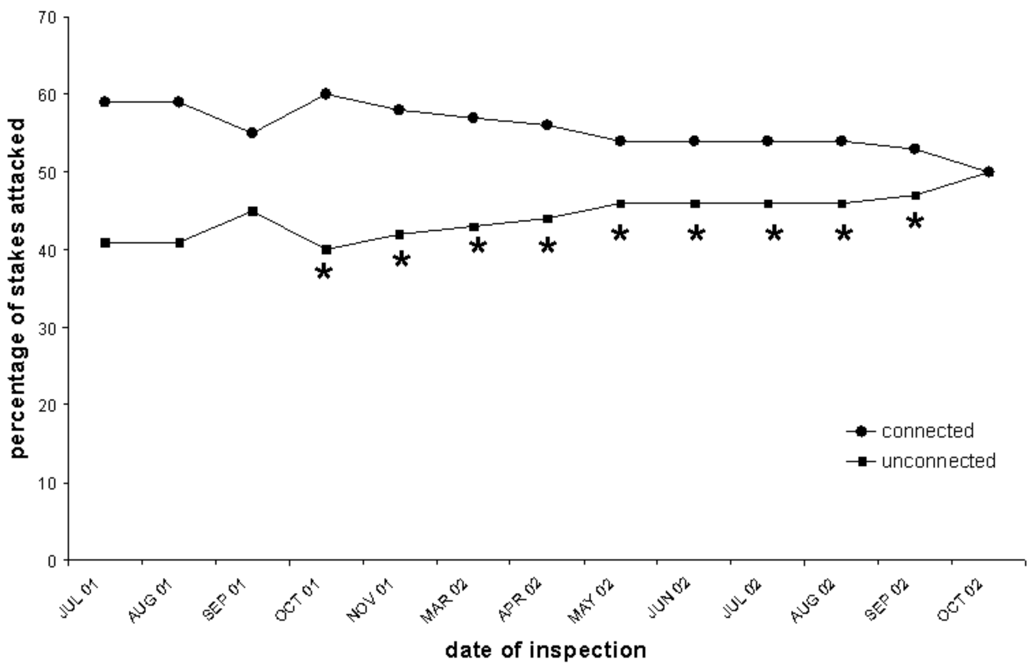

Figure 4.9. Proportion of connected and unconnected stakes attacked at Hare Road Farm over time**.** Proportion of stakes connected by guidelines and attacked was significantly different from proportion of unconnected stakes attacked on dates designated by an asterisk (*).

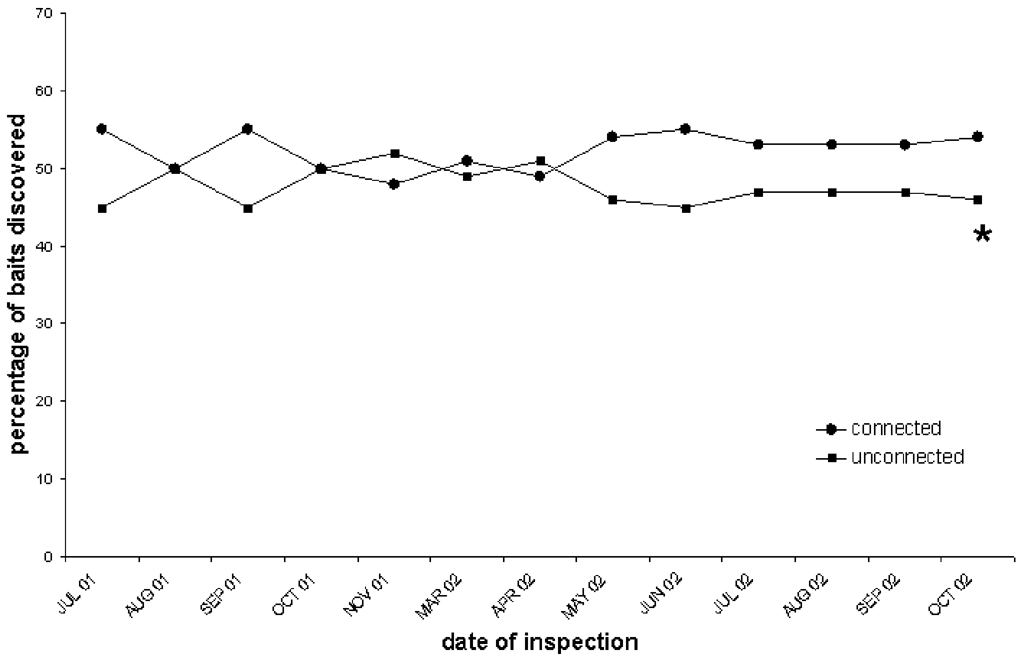

Figure 4.10. Percentage of connected and unconnected stakes attacked at Mason Neck State Park over time. Proportion of stakes connected by a guideline and attacked was significantly difference from proportion of unconnected stakes attacked on dates designated with an asterisk (*).

At Mason Neck State Park, no significant difference was found in the proportion of connected and unconnected stakes attacked during the field study. However, when the stakes were destructively examined at the end of the study, significantly more stakes connected by physical guidelines had been attacked by *Reticulitermes* spp. (Table 4.10; 54%; χ^2 = 3.83, df = 1, P= 0.05).

Discussion

Several field studies have suggested that subterranean termites attack food items located on the soil surface more frequently than they attack similar items buried in the soil (Santos, 1979; Ettershank et al., 1980). In another field study, French (1991) found that baits located below the soil surface were more likely to be discovered by several economically important Rhinotermitidae when positioned within the root systems of woody plants. One explanation for increased discovery of baits proximal to roots is simply that roots and fallen plant material are important food resources for termites. Following roots may also enhance foraging success by leading subterranean termite workers to fallen wood below the canopy of a tree or shrub. King and Spink (1969) observed subterranean termites constructing tunnels directly below living roots on which they were not feeding. Such behavior has been identified as guideline following (Jander and Daumer, 1974).

There are numerous anecdotal accounts of termites following pipes, conduit and other inorganic guidelines into structures in much the same manner that they follow roots. Mud tubes connecting structural termite infestations to the soil commonly follow cracks, pipes and other architectural guidelines (Potter and Hillery, 2001; Beard, 1974). In addition, two commercial subterranean termite baiting systems have incorporated plastic guidelines, meant to guide foraging termites to the bait matrix, into their bait station designs.

Several investigators have experimented with the use of physical guidelines to direct termite foraging behavior in laboratory bioassays. Pitts-Singer and Forschler (2000) found that *R. flavipes* would follow horizontally oriented wires through the substrate. Campora and Grace (2001) found that tunneling by subterranean termites increased in the area of physical guidelines or 'anomalies' in the substrate, whether or not there was cellulose present.

If the subterranean termite populations at our test sites followed the physical guidelines deployed in test plots to the bait stakes, the effect was not strong. The results of our field study were inconclusive. Our data do not indicate that connecting bait stations with artificial physical guidelines increased the probability of bait discovery.

In Chapter III, we demonstrated that *Reticulitermes* spp. would follow physical guidelines under laboratory conditions. Why were our field results inconclusive? In the laboratory bioassay arenas, the guidelines were a unique artifact in an otherwise homogenous environment. The termites' centered their activity around the guidelines because there were no other orientation cues.

Under field conditions, foraging termites encounter many environmental influences including variations in available moisture, thermal shadows, predators and potential food items. It seems likely there is a hierarchy of stimuli that affect the termites' foraging and feeding activity. The influence of a physical guideline may be less important than the stimulus of a significant thermal shadow or a chemical cue. Guideline following was documented by King and Spink (1962), as one foraging strategy of subterranean termites, but it is not the only strategy termites use to locate food. When we attempted to direct termite movement through the soil with guidelines under field conditions guidelines did not significantly improve bait location.

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CHAPTER V: THE EFFECT OF THERMAL SHADOWS ON FORAGING SUBTERRANEAN TERMITES

Introduction

Subterranean termites (Isoptera: Rhinotermitidae) typically forage beneath the soil surface but are able to identify areas where food is plentiful above ground (King and Spink, 1969; Wood, 1978a, b; Shahid and Akhtar, 1989; Reinhard et al., 1997). For example, Gentry and Whitford (1982) found that the density of foraging subterranean termites in coastal habitats was positively correlated with the amount of wood litter available on the soil surface. Haverty and Nutting (1975) found that desert subterranean termites also aggregated in habitats where fallen dead wood was abundant.

The mechanism that allows termites, foraging underground, to detect food lying on the soil surface is unclear. It is interesting to note that subterranean termites attack food items, including toilet paper rolls, boards, newspapers, and bags of leaf litter located on the soil surface more frequently than they attack and consume similar resources buried underground. Apparently, subterranean termites are better able to locate food resources on the soil surface than items that are buried (Santos, 1979; Ettershank et al., 1980; Shahid and Akhtar, 1989). Objects on the surface of the soil create thermal shadows in the substrate beneath them when the heat capacity of the object (ability to absorb and store heat energy generated by the sun) is different from the heat capacity of the surrounding soil. In general, objects with high moisture content have a high heat capacity and create a cool thermal shadow (Ettershank et al., 1980; Smith and

Rust, 1994; Long et al., 2001). Objects with lower moisture content than the surrounding soil, like rocks or metal, have a low heat capacity. Objects with a low heat capacity can transmit heat energy into the soil creating warm thermal shadows (Danalatos et al., 1995). It has been speculated that termites may be able to detect objects on the soil surface by sensing such thermal shadows (Ettershank, 1980).

Substrate temperature is known to directly affect termite behavior, including feeding (Fei and Henderson, 2002; Rust et al., 1996; Cabera and Rust, 1994; DeLaplane et al, 1991), tunneling (Cabera and Kamble, 2001; Strack and Myles, 1997; Collins, 1991; Ettershank et al., 1980), and aggregation (Cabera and Rust, 1996). It is possible that increased discovery of food items is coincidental to increased tunneling areas sheltered beneath surface features. Gallery building may be increased in sheltered areas where the soil is shielded from extremes of heat and cold (Ettershank et al., 1980; Smith and Rust, 1994; Potter et al., 2001; Forschler, personal communication). However, the variations in soil temperature under potential food resources may trigger more specific changes in termite foraging activity.

Rapid construction of straight galleries through the substrate is a characteristic behavior of subterranean termites searching the environment for food (Robson et al., 1995; Reinhard et al., 1997; Puche and Su, 2001a, b). When foraging subterranean termites encounter a food resource, they build a dense network of short tunnels around it in an apparent effort to explore its potential as food (Goldberg, 1973a; Williams, 1977; Reinhard et al., 1997).

Encountering a thermal shadow in the substrate may also lead foraging termites to cease building straight exploratory tunnels and begin exploratory tunneling within the thermal shadow (Ettershank et al., 1980).

If subterranean termites are attracted to thermal shadows it might be possible to direct their foraging behavior by creating "spot" of differing temperature within a homogenous substrate. However, an evaluation of subterranean termite response to thermal shadows has never been performed under laboratory conditions. This study was an attempt to determine whether subterranean termites are attracted to thermal shadows or simply aggregate to areas of optimum temperature.

Materials and Methods

Subterranean termites. *Reticulitermes* spp. were collected from wild populations and maintained at the Dodson Urban Pest Management Laboratory (DUPML) at Virginia Tech in Blacksburg, Virginia. Termites were harvested from naturally infested wood collected on roadsides and in residential and forested areas in the Piedmont, Coastal Plain, and Ridge and Valley geological provinces of Virginia. Termites were extracted by placing infested wood on damp paper towels (Prolink™, San Antonio, Texas) with open ends of termite galleries contacting the paper. The wood was allowed to air dry. As the wood desiccated, the termites left the wood and entered the damp paper. Once the paper became infested it was removed and placed in a plastic storage container (15cm x 12cm x 30cm; Newell Rubbermaid Inc., Freeport, IL) containing vermiculate (Morton's Horticultural Products, McMinnville, TN) and white pine blocks as a food source (Heavener's Hardware, Blacksburg, VA). Fresh paper was placed under the infested wood to extract additional termites. When termite recruitment to the paper towels slowed or ceased, the wood was opened with a hatchet and the remaining termites were shaken into the plastic rearing container holding the termites from the same piece of wood. Containers of termites were stored in unlighted wooden cabinets and maintained between 20 and 28 °C. No attempt was made to control the relative humidity in the storage area. When the paper in the storage boxes appeared to be dry, 25 ml of water was poured onto the paper towels to dampen them.

Thermogradient table. A two-dimensional thermogradient table was used to obtain a continuous temperature gradient (Fig. 5.1;Fig. 5.4; Designed by Dr. Greg Welbaum, Department of Horticulture, Virginia Tech and constructed by Dr. John Cundiff, Department of Biological Systems Engineering, Virginia Tech). The table consisted of a hollow aluminum box (90 cm x 120 cm x 7.5 cm) containing a loop of copper pipe at each end. The intake and output valve for each loop of pipe were connected with plastic tubing (New Age Industries, Inc. Southampton, PA) to a Lauda RM 6 heating /cooling circulating bath (Brinkman Instruments, Inc., Westbury, NY) to create a circulating system. By circulating liquid of different temperatures through two circulating systems, one at each end of the table, a stable temperature gradient could be maintained across the long axis of the table. The intake valve for each loop of copper pipe is fitted with a regulator so that the rate of flow through the circulating system may be adjusted. The cold circulating system contained Xerex Antifreeze (50%; The Valvoline Company, Lexington, KY). The hot circulating system contained tap water.

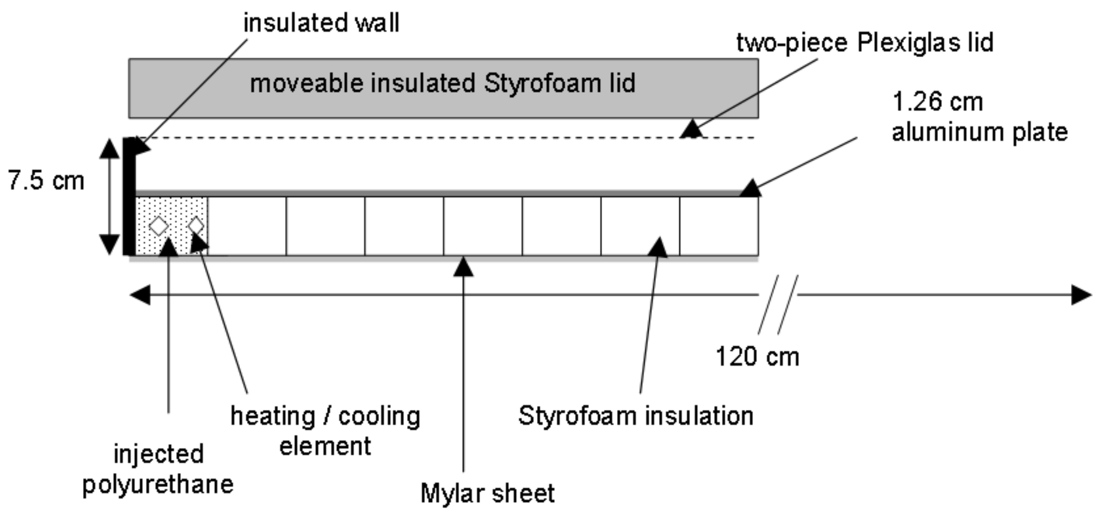

Figure 5.1. Side view of the components of the thermogradient table used in the bioassay to determine preferred substrate temperature for *Reticulitermes* spp (Greg Wellbaum, personal communication.).

Temperature-controlled reservoirs. Thermal shadows were created using a temperature-controlled reservoir, which consisted of a 500 ml plastic storage container (Dollar Tree Distributors, Chesapeake, VA) with a tight fitting lid. Two 0.8 cm holes were drilled in the lid of each reservoir (Fig. 5.2 A) and a 0.5 m length of plastic tubing (New Age Industries, Inc. Southampton, PA) with a 0.75 cm aperture was laced through the two holes. Four hundred milliliters of tap water was added to the plastic storage container and the reservoir was sealed

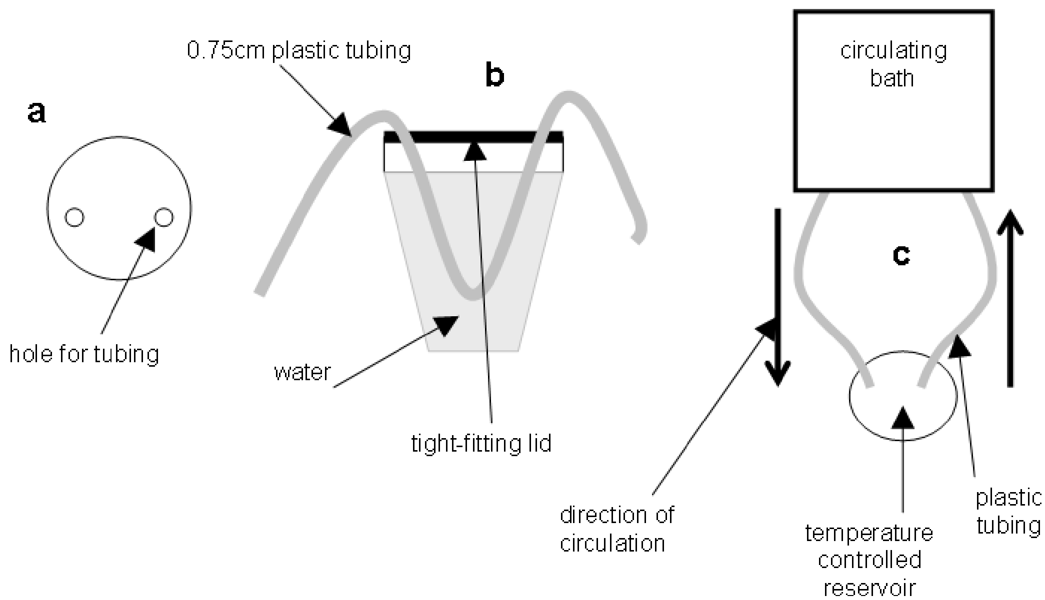

Figure 5.2. Design of the temperature controlled reservoir. a. Top view of lid showing holes for insertion of tubing. b. Side view of the reservoir. c. Top view of the reservoir attached to the circulating bath showing direction of water circulation.

with the lid fitted with plastic hosing (Fig. 5.2 B). The plastic hose for the cooled temperature-controlled reservoir was connected to a Lauda RM6 circulating bath (Brinkman Instruments Inc., Westbury, NY). The plastic hose for the heated temperature-controlled reservoir was connected to a Haake KT2 circulating bath (Thermoelectron, Corp., Newington, NH). By circulating heated or cooled water through the reservoir a stable temperature, differing from ambient room temperature could be maintained in the substrate below the reservoir (Fig. 5.2 C). The temperature of the circulating bath attached to the temperature-controlled reservoir was adjustable.

Thermometers. Temperatures within bioassay arenas for the two assays described in this chapter were measured using Accurite 667 cooking thermometers (Cheney Instrument Company, Lake Geneva, WI). These thermometers are equipped with a remote thermocouple probe on a flexible metal cable (100 cm), which allowed us to record temperatures within sealed bioassay arenas. Thermometers were calibrated using boiling water (100 C°) and found to be accurate to $\pm$ 1 °C. During the bioassay to determine the effect of thermal shadows on termite aggregation, room temperature was measured using a THGR238 Thermo-Hygrometer (Oregon Scientific, Tualatin, Oregon).

Bioassay to determine the preferred substrate temperature for *Reticulitermes* spp. Bioassay arenas consisted of Plexiglas boxes (3 cm x 2 cm x 90 cm) with lids. Each arena contained three thermocouple probes affixed to the junction of the floor and inner wall of the box with modeling clay (Dixon Ticonderoga Corp., Sandusky, OH) one probe was located 5 cm from each end of the arena and one at the center of the arena (Fig. 5.3).

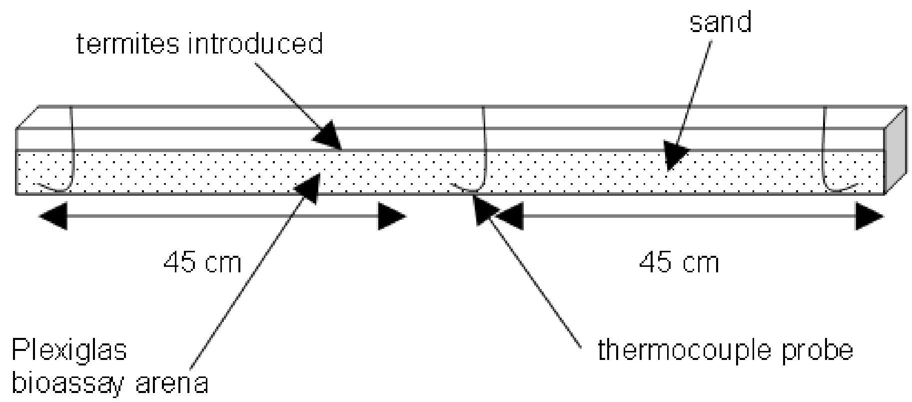

Figure 5.3. Diagram of the arena for the bioassay to determine the preferred substrate temperature.

Sand (Playsand, Quikcrete™ Corporation, Atlanta, GA) was separated into 750 mg units. Aliquots of sand were stored in one-quart plastic storage bags. Tap water (112.5 ml; 15% by weight) was added to each bag of sand. The bag was sealed and kneaded by hand to mix sand and water evenly. Moistened sand was placed into the bioassay arena and spread evenly over the thermocouple probes and the bottom of the arena.

Bioassay design. Prior to testing, a population of termites (150 workers) was removed from a rearing container with an aspirator and transferred into a plastic Petri dish (4 cm, Becton Dickinson Labware, Lincoln Park, NJ). A bioassay arena, containing thermocouple probes and sand was positioned on the heat gradient table perpendicular to the heating/cooling elements (Fig. 5.4). The cool bath was set at –30 °C and the hot bath was set at 45 °C. The flow rate for

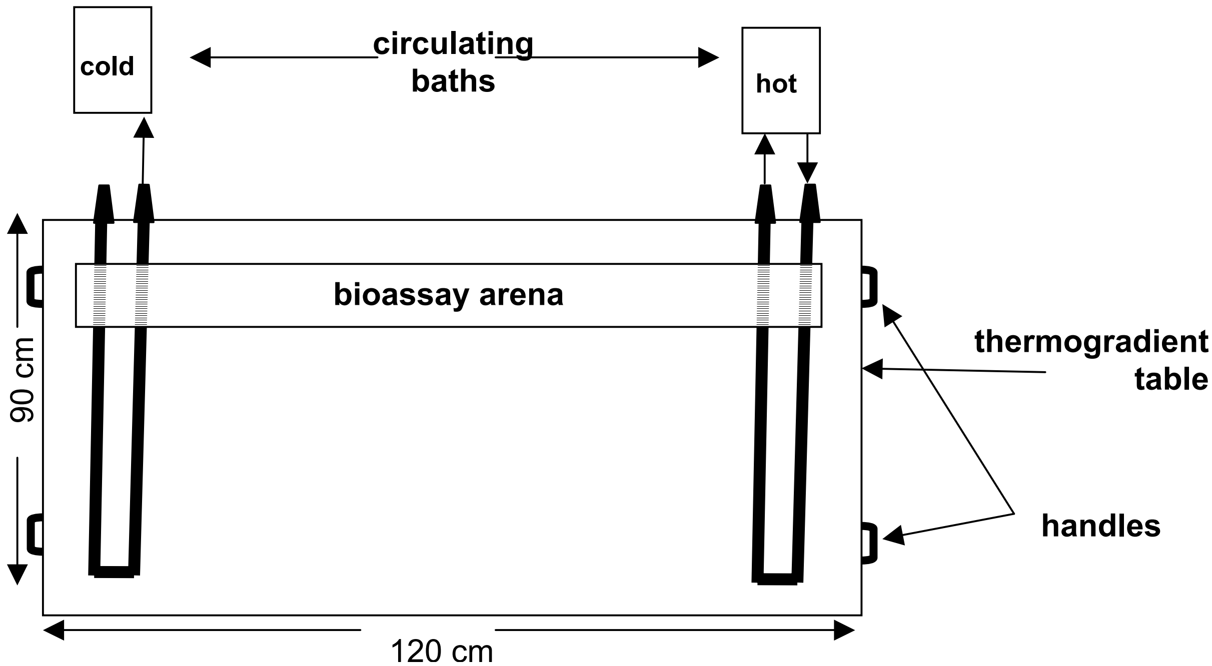

Figure 5.4. Top view of the thermogradient table showing the position of the bioassay arena (Greg Welbaum, personal communication).

the cool circulating system was adjusted to ~ 2 gallons/minute. The flow rate for the hot circulating system was adjusted to ~ 1 gal/minute. An insulating lid was placed over the cool circulating system and the bioassay arena (Fig. 5.1). This arrangement created a temperature gradient between 10 °C and 38 °C within the bioassay arena, with a mean temperature change of 1°C / 4 cm. The temperature of the table's surface along a transect perpendicular to the temperature gradient varied approximately ±1 °C. The entire surface of the table was covered with black felt to exclude light. The thermogradient table was allowed to run for approximately one hour to stabilize the temperature gradient within the arenas prior to introduction of termites.

Once the desired temperature gradient was achieved for a period of 20 minutes, the termites were introduced into the arenas by tapping them out of the Petri dish and onto the surface of the sand. The population of termites was introduced into the bioassay arena at the point where the temperature of the substrate was ~20° C. The insulating lid was put on the cool end of the table after the termites were introduced and the entire table and arenas were covered with black felt to exclude light. The termites were allowed to forage *ad libitum* for 24 h. After 24 h, the temperatures of the thermocouple probes in the unopened bioassay arena were checked and recorded. If the temperatures within an arena varied by more than 2 °C from the temperature recorded at the time of termite introduction, the assay was aborted. If the temperatures were within acceptable limits, the bioassay arenas were suspended over a mirror. The position of the termites within the bioassay arena was clearly visible through the Plexiglas bottom. Additional thermocouple probes were inserted into the sand at the outer edges of the visible termite activity and the thermogradient table was closed. The bioassay arena remained on the thermogradient table for an additional 30 minutes. Temperature readings were taken from the thermocouple probes at the edges of the termite activity and the temperatures were recorded. After temperatures were recorded, the bioassay arena was inverted over a row of five Nunc™ bioassay dishes (23 cm x 23 cm x 2 cm; Nalge Nunc International, Rochester, NY) and the sand was examined to confirm the termites' position in the arena. The assay was replicated seven times.

Bioassay to determine the effect of thermal shadows on termite aggregation. Bioassay arenas consisted of Nunc™ bioassay dishes (23 cm x 23 cm x 2 cm; Nalge Nunc International, Rochester, NY). Each arena contained four brown, recycled, single fold paper towels (Prolink™, San Antonio, Texas) folded into 7cm x 7cm squares and saturated with 5 ml tap water. The folded towels were positioned in the bottom of a bioassay arena with the edges parallel to the other towels approximately 4 cm apart and approximately 2.5 cm from the edge of the Nunc™ dish. A thermocouple probe was inserted into the towel covered by the temperature-controlled reservoir. A second thermocouple probe was inserted in the towel covered by the empty plastic container to measure the ambient temperature in the rest of the arena (Fig. 5.5). The entire dish was wrapped in aluminum foil (Reynolds Metals Co., Richmond, VA). A temperature-controlled reservoir (Fig. 5.2) was positioned over one of the folded paper towels. The other three paper towels served as controls. A snap-top 500 ml plastic storage container (Dollar Tree Distributors, Chesapeake, VA) holding 400 ml of tap water at ambient room temperature was positioned over one control paper towel; an empty 500 ml storage container was positioned over the second control paper towel. The third control paper towel was left uncovered (Fig. 5.6). The entire arena was covered with a black cloth to exclude light. The circulating baths were allowed to run for approximately one hour to permit the temperatures under the temperature-controlled reservoir and in the rest of the arena to stabilize. Once the temperatures had remained stable for 20 minutes the thermocouple probes were removed and the bioassay was begun.

Bioassay design. Two populations of 150 termite workers each were aspirated from the same colony. One population was introduced into the center of the arena containing the warm temperature-controlled reservoir and one population was introduced into the center of the arena containing the cool temperature-controlled reservoir. The termites were allowed to forage *ad libitum* for 2 h. After the foraging period, the bioassay arenas were opened. Each folded paper towel was placed into a separate Nunc™ bioassay dish (23 cm x 23 cm x 2 cm; Nalge Nunc International, Rochester, NY). Each of the paper towels was unfolded and the termites aggregated on the paper towel were counted (Fig. 5.7).

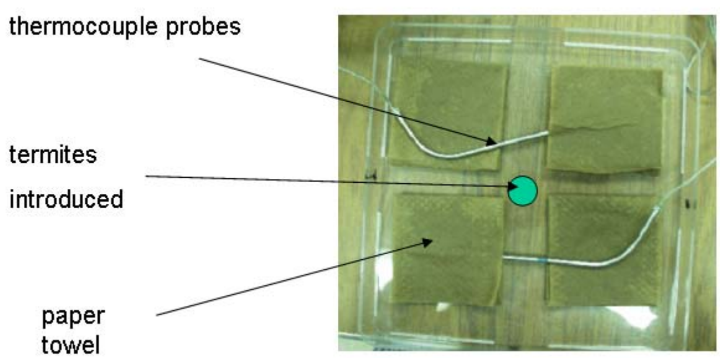

Figure 5.5. Bioassay arena used in the bioassay to determine the effect of thermal shadows on termite aggregation.

Figure 5.6. Bioassay arena used in the bioassay to determine the effect of thermal shadows on termite aggregation.

Statistical analysis. Temperatures were calculated for high, low and preferred temperatures for the bioassay data on preferred substrate temperature for *Reticulitermes* spp. (Microsoft Excel, 2002). For the thermal shadow studies, the proportion of termites aggregating on each folded paper towel relative to the total number of termites aggregating all four paper towels in an arena was compared. Within each treatment, χ^2 tests were performed based on the generalized linear models (PROC GENMOD, SAS Institute, 1999). P values of $\leq$ 0.05 indicated significance.

Figure 5.7. Termites aggregated in a paper towel could be counted when the towel was unfolded.

Results and Discussion

Bioassay to determine the preferred substrate temperature for Reticulitermes spp. The preferred substrate temperature range for *Reticulitermes* spp. workers in this assay was 18 - 27 °C. The mean preferred temperature was 22.5 °C. The optimum temperature for laboratory maintenance of *R. flavipes* reported by Becker (29 °C; 1970a) was slightly warmer than the preferred range observed in our assay.

Our recorded preferred temperatures are somewhat higher than those documented in several other experiments (Smith and Rust, 1994; Houseman et al., 2001). Smith and Rust (1994) found that *R. hesperus*, the western subterranean termite, when exposed to a temperature gradient between 5.8 and 43.6 °C in a laboratory bioassay aggregated between 14 and 19 °C. *Reticulitermes hesperus* preference for cooler temperatures may be an adaptation evolved to minimize moisture loss in its native desert environment. In a field study of subterranean termite activity in Texas, Houseman et al. (2001) found that *R. flavipes* was significantly less active in July, when soil temperatures were between 25 and 30 °C, than in January when soil temperatures were between 10 and 15 °C. The differences in temperature preference of subterranean of termites in Texas and those used in this study may simply indicate that populations of *Reticulitermes* spp. acclimate to local conditions.

Even within a species, different populations may have different behaviors and preferences. Becker (1970b) reported that populations of *R. flavipes,* collected in northern locations, built stronger galleries and had more pronounced daily rhythms of activity than *R. flavipes* collected further south. In addition, Becker found that populations of *R. flavipes* collected in the south fed more voraciously at lower temperatures than conspecifics collected further north.

The difference in observed temperature preference reported in these four studies might also be due to differences in experimental design. Reticulitermids are incapable of conserving body moisture when exposed to dry air (Collins, 1969, 1991; Rudolph et al., 1990). Social control of nest humidity is a prominent feature of the subterranean termite's survival adaptations (Collins, 1969). Several previous studies have demonstrated that subterranean termites tunnel preferentially in areas where the moisture content of the substrate and hence the level of humidity in the soil atmosphere is within certain limits (Powell, personal communication; Puche and Su, 2003).

In our bioassay to determine the preferred substrate temperature for *R. flavipes*, subterranean termites were maintained in moist substrate during the experiment and the bioassay arenas were sealed to minimize moisture loss. Becker (1970a) used a similar experimental design where moisture was sealed in the arena. In both studies, the subterranean termites tunneled into the substrate upon release, and created sealed galleries where they could maintain the humidity at their preferred level. With the humidity held constant, the effects of temperature could be observed without any confounding effects due to moisture loss. In Houseman et al.'s (2001) field study, soil moisture content dropped as the temperature rose. The authors postulated that the termites might have been more active when the soil was cooler to minimize moisture loss.

In Smith and Rust's (1994) laboratory bioassay *R. hesperus* workers were exposed to a temperature gradient in a sealed assay arena on damp paper. Artificially humidified air (90% humidity) was pumped into the arena. In spite of the fact that a high humidity level was maintained in the arena, the termites were not provided with substrate to create galleries and control the relative humidity of the air around them. Fluctuations in humidity may have occurred. In addition, 90% humidity may have been lower than their optimum level thus triggering movement to the cooler portion of the gradient. Rudolph et al. (1990) found that *R. lucifugus* lost up to 10% of their body mass due to desiccation when maintained at 98% atmospheric humidity for 24 hours. Smith and Rust (1994) observed that the percentage of termites aggregating in the cooler portion of the arena increased significantly when the atmospheric humidity was reduced to 20% and postulated that minimization of moisture loss was a factor in the behavior of R*. hesperus*. When the subterranean termites were provided with sand to construct galleries, they were able to control their atmospheric humidity level, Becker's assay and our bioassay probably represent the most accurate picture of *Reticulitermes* spp. substrate temperature preferences.

Bioassay to determine the effect of thermal shadows on termite aggregation. Subterranean termite aggregation was significantly influenced by the presence of thermal shadows. Termites were significantly more likely to aggregate in cool thermal shadows (χ^2 = 27.64, df = 3, $\underline{P}$ = 0.01) than in controls at ambient air temperature. The mean number of *Reticulitermes* spp. workers aggregated in warm thermal shadows did not differ significantly (χ^2 = 6.67, df = 3, $\underline{P}$ = 0.08) from the mean number aggregated in controls (Table 5.1).

Reticulitermes spp. workers were significantly more likely to aggregate in a 20 °C thermal shadow (χ^2 = 17.3, df = 3, $\underline{P}$ = 0.02), than in controls at ambient room temperature (25 °C). While 20 °C is cooler than the mean preferred temperature for *Reticulitermes* spp. (22.5 $\pm$ 3 °C), it lies within the preferred temperature range (18 - 27 °C).

A second assay was run to determine if *Reticulitermes* spp. would aggregate to a cool thermal shadow outside of their preferred temperature. *Reticulitermes* spp. workers were significantly more likely to aggregate in thermal shadows of 15 °C (χ^2 = 10.25, df = 3, $\underline{P}$ = 0.01) than in controls at room temperature, 20 °C (Table 5.1). The termite's aggregation in the cool (15 ° C) thermal shadow is particularly interesting because 15 °C lies outside of their preferred temperature range while the temperature of the surrounding arena (20 °C) was with in the preferred range.

The fact that we found that subterranean termites would aggregate in cool thermal shadows supports the contention of Ettershank et al. (1980) that subterranean termites locate food on the soil surface by sensing thermal shadows in the substrate. Subterranean termite aggregation in cool thermal shadows also explains the observation made after several earlier field studies that subterranean termites attacked potential food items located on the soil surface, but not similar food items buried nearby (Santos, 1979; Ettershank et al., 1980; Sahid and Akhtar, 1989). The fact that *Reticulitermes* spp. workers aggregated in cool thermal shadows but not in warm thermal shadows suggests

Table 5.1. Proportion of subterranean termites aggregating within a thermal shadow compared with the proportion of termites aggregating within control areas of the bioassay arena (df = 3).

Control Temp.	Thermal Shadow Temp.	Mean Percent (± SEM) of Termites Aggregating[5]					n	χ^2	P
		In Arena	Thermal Shadow[1]	Cont. a[2]	Cont. b[2]	Cont. c[3]			
Cool Thermal Shadow			**51±10a**	**14 ± 6b**	**24±9b**	**10±6b**	**23**	**27.6**	**0.001**
20° C	**15° C**	63±7	54±13a	18±9b	20±1b	8±2b	16	17.3	0.01
25° C	**20° C**	78±7	58±14a	12±10b	26±11b	14±10b	10	10.3	0.02
Warm Thermal Shadow			**17±6a**	**42±12a**	**20±9a**	**21±8a**	**20**	**6.7**	**0.08**
20° C	**25° C**	66±17	16±6a	33±16a	20±16a	16±16a	10	1.5	0.67
25° C	**30° C**	62±16	19±11ab	50±18a	20±9ab	10±5b	10	8	0.05

[1] A folded paper towel under a temperature-controlled reservoir.

[2] A folded paper towel under a plastic container holding 400 ml of water at ambient room temperature.

[3] A folded paper towel under an empty plastic container at ambient room temperature.

[4] A folded paper towel at ambient room temperature.

[5] Mean temperatures ± SEM are significantly different at P = 0.05. Means ± SEM followed by different letters are significantly different.

that subterranean termite foragers can discriminate between probable food resources and inedible surface features like stones.

Organic objects on the soil surface like wood and animal carcasses tend to have high moisture content and may absorb environmental moisture (Corey and Kemper, 1968). Organic materials have a high heat capacity, and therefore they warm slowly when exposed to solar radiation and cool slowly when no external source of heat is present. Thus, organic objects on the soil surface act as heat sinks (objects that radiate heat at a slower rate than the surrounding substrate). The temperature of the soil below organic features is buffered from changes in the atmospheric temperature (Ettershank et al., 1980; Haywood et al., 1997). During the day, an organic object, like a log, located on the soil surface creates a cool thermal shadow below it in the soil. Thus, cool thermal shadows are likely to indicate the presence of a food resource for subterranean termites. By exploring cool thermal shadows subterranean termites are likely to discover nutritional resources like wood or other edible organic matter (Fig. 7.1).

In contrast, mineral features on the soil surface (rocks) have low moisture content and low heat capacity. Rocks heat rapidly when exposed to solar radiation and cool rapidly as well (Danalatos et al., 1995). When the surface of the soil contains medium sized rocks or cobbles, rounded particles between 64 and 256 mm in size (Anonymous, 1988), changes in atmospheric temperature are rapidly transmitted to the soil through these rocks (Danalatos et al., 1995). Cobbles are rapidly warmed by the sun and create warm thermal shadows in the soil directly beneath them. Warm thermal shadows are likely to indicate an inedible obstruction on the soil surface.

Several researchers have observed *Reticulitermes* spp*.* aggregating under stones in the field (Smith and Rust, 1994; B. T. Forschler, personal communication). This observation does not necessarily contradict the finding that termites prefer cool thermal shadows and ignore warm thermal shadows. The termites may simply have been taking advantage of gaps formed beneath the stones by bioturbation (movement of the substrate by living organisms), thermoturbation (movement of the substrate resulting from changes in temperature) or changes in soil moisture. In addition, a large stone might help to retain soil moisture in the sheltered area making the substrate beneath it attractive to subterranean termites in the heat of summer (Smith and Rust, 1994). Although their heat capacity is low, large stones can create cool thermal shadows. Large stones have a low specific surface area, meaning that the ratio of surface area to mass is higher than the ratio of smaller stones (Brady and Weil, 1999). The portion of the stone exposed to solar energy is lower than for smaller stones, so the overall temperature of the stone rises more slowly. Until sufficient energy is absorbed to heat its entire mass, a large stone shelters the substrate beneath it, creating a cool thermal shadow (Smith and Rust, 1994). Thus, subterranean termites, aggregating under stones, may sometimes be exploring a cool thermal shadow.

The termite response to thermal shadows has interesting implications for termite bait station design. If termites detect food on the soil surface by locating cool thermal shadows in the soil, then designing a bait station that creates a cool thermal shadow should facilitate termite aggregation at the station and subsequent bait discovery. In spite of the fact that several researchers have demonstrated that subterranean food items are less likely to be discovered than nutritional resources located on the soil surface (Santos, 1979; Ettershank et al., 1980, Sahid and Akhtar, 1989), all subterranean termite bait stations currently marketed in North America consist of plastic containers buried in the substrate with nothing more than a thin ring of plastic at the soil surface. Such designs do not create a significant thermal shadow. Further research should be conducted to determine how bait station design could be altered to create a significant cool thermal shadow around the bait.

CHAPTER VI: THE EFFECT OF NUTRIENT COMPOUNDS (SUGARS AND AMINO ACIDS) ON BAIT CONSUMPTION BY RETICULITERMES SPP.

Introduction

In 1994, DowElanco introduced the first commercially available subterranean termite baiting system (Quarles, 1995; Robertson and Su, 1995; Su et al., 1995). This product was labeled the Sentricon Termite Elimination System™ and its appearance on the market was followed shortly by competing bait systems FirstLine™ and the Exterra™ (Quarles, 1995; Pawson and Gold, 1996; Su and Scheffrahn, 1998). Bait systems for termites have been very appealing to consumers who are concerned about environmental contamination and potential pesticide exposure. The reason for this appeal is that bait toxicants have very low mammalian toxicity and are confined within a station where they have almost no potential for environmental contamination (Jones et al., 1987; French, 1991; Quarles, 1995; Pawson and Gold, 1996; Potter et al., 2001; Verkerk and Bravery, 2001). Baiting systems are especially suitable for use around homes near bodies of water or that have wells within close proximity to the structure (Robertson and Su, 1995; Su, 1997; Su and Scheffrahn, 1998). Subterranean termite baiting technology has been a major breakthrough in termite management and baiting systems have quickly captured a significant portion of the termite control market (Pawson and Gold, 1996; Su and Scheffrahn, 1998; Potter et al., 2001).

Subterranean termite baiting systems attempt to exploit the natural foraging and food distribution behavior of termites to introduce the bait toxicant into the colony (Olstaff and Gray, 1975; Esenther and Beal, 1979; French, 1991; Su et al., 1995). In order for a subterranean termite baiting system to work, foragers must recruit to the bait in large numbers and consume a sufficient quantity of bait to affect a significant portion of the population. The termites must then return to the nest to distribute the toxicant throughout the colony (Esenther and Beal, 1979; Su et al., 1987; Su, 1995; Grace et al., 1996a; Su and Scheffrahn, 1996).

Although the exploitation of termite behavior as a delivery system for a bait toxicant is an excellent idea, this methodology faces several challenges in the field. Pawson and Gold (1996) noted that wild populations of subterranean termites often find wood stakes more palatable than the bait matrices in commercial bait systems. If subterranean termites find the bait matrix less palatable than other food resources in the same area, the efficacy of the bait system can be greatly reduced. In other words, if foragers do not consume a sufficient quantity of the bait there will not be enough toxicant introduced into the colony to significantly impact the population. In addition, if baits are not sufficiently palatable to compete with other food resources, they are likely to be abandoned once a more desirable source of food is discovered. A third challenge for such systems is that baits have to be replenished periodically. Because subterranean termites are sensitive to disturbance, the replacement or replenishment of the bait matrix in an active station can cause termites to abandon the bait station and not return (Nutting and Jones, 1990; Su and Scheffrahn, 1996; Su, 2001).

Ideally, a termite bait matrix would be more palatable than other food resources in the area, causing termites to feed preferentially on the matrix, even when non-toxic food sources are available. Because it is well documented that subterranean termites prefer foods that contain certain nutrients (Smythe and Carter, 1970; French et al., 1986; Grace et al., 1986; Waller, 1988; Delaplane, 1989; Oi et al. 1996; Doi et al., 1999), it is reasonable to suggest that a particular nutrient, or group of nutrients could be added to a termite bait matrix to enhance its palatability for termites. Several sugars and nitrogen compounds have already been evaluated for their ability to influence bait consumption in *Reticulitermes spp.* and *Coptotermes formosanus* (Mishra, 1992, Chen and Henderson, 1996, Waller and Curtis, 2003). However, these studies were not comprehensive, evaluating less than 5 nutrients for *Reticulitermes spp*. Consequently, the commercial bait systems currently available contain no supplementary nutrients or feeding stimulants to enhance bait consumption.

The series of bioassays described in this chapter expanded on a study conducted by Waller and Curtis (2003) who found that the addition of certain sugars to a bait matrix (filter paper) caused *Reticulitermes spp*. feed on the matrix preferentially. The objectives of these assays were to test a variety of sugars in choice and no-choice tests to evaluate their effect on termite bait consumption. In addition, because nitrogen compounds are extremely limited in the subterranean termite diet (Hingate, 1941; Potrikus and Breznak, 1981;

Waller, 1988; Reinhard and Kaib, 2001) several amino acids and uric acid were evaluated to determine their potential as termite feeding stimulants or deterrents.

Materials and Methods

Subterranean termites. Reticulitermes spp. were collected from wild populations and maintained at the Dodson Urban Pest Management Laboratory (DUPML) at Virginia Tech in Blacksburg, Virginia. Termites were harvested from naturally infested wood collected on roadsides or in residential and forested areas in the Piedmont, Coastal Plain, and Ridge and Valley geological provinces of Virginia. Termites were extracted by placing infested wood on damp paper towels (Prolink™, San Antonio, Texas) with the open ends of the termite galleries contacting the paper. The wood was allowed to air dry. As the wood dried, the termites left the wood and entered the damp paper. Once the paper became infested, it was removed and placed in a plastic storage container (15cm x 12cm x 30cm; Newell Rubbermaid Inc., Freeport, IL). Fresh paper was placed under the wood to extract additional termites. When termite recruitment to the paper towels slowed or ceased, the wood was opened with a hatchet and the remaining termites were shaken into a plastic rearing container holding termites from the same piece of wood. Containers of termites were stored in unlighted wooden cabinets and maintained between 20 and 28°C. No attempt was made to control the relative humidity in the storage area. When the paper in the storage boxes appeared to be dry, 25 ml of water was poured onto the paper towels to dampen them.

Test nutrients. Sixteen nutrient compounds including sugars, amino acids, and uric acid, were evaluated for their ability to affect termite feeding. (Table 6.1). Three of the sugars selected for testing (glucose, xylose and sucrose) were those evaluated by Curtis and Waller (2003) and found to be termite phagostimulants. Fructose, raffinose and galactose were selected as test nutrients because they are all important components of woody plant tissue, the principle food of *Reticulitermes spp*. (Kaar and Brink, 1991; Ashworth et al., 1993; Wong et al., 2003). Trehalose was selected because many insects including termites, store carbohydrates in the form of trehalose (Chapman, 1998). Arbutin, a phenyl sugar, was selected because it is the chemical precursor of hydroquinone, a powerful phagostimulant for the order Isoptera (Reinhard et al., 2002).

Because nitrogen compounds had not been evaluated for their influence on *Reticulitermes spp*. feeding, but are known to be phagostimulants for *Coptotermes formosanus* (Chen and Henderson, 1996) a variety of amino acids were selected for their high water solubility and ease of application to the bait matrix. Uric acid was selected for evaluation because *Reticulitermes spp*. is known to sequester nitrogen in its fat body as uric acid (Potrikus and Breznak, 1981).

A 0.1 molar stock solution of each nutrient compound was prepared by dissolving each compound (laboratory grade) in distilled water. Additional, 0.01 M and 0.001 M solutions were prepared from the stock solution. Due to low solubility of uric acid, it was not possible to formulate a true solution at the higher concentrations of 0.1 and 0.01 M. Therefore only the 0.001 M solution was evaluated in this assay.

Termite bait matrix. The termite bait matrix consisted of 2 cm circles of brown paper towel (Prolink™, San Antonio, Texas). Each bait was placed on a microscope slide cover slip (18 x 18 mm; Fisher Scientific, Pittsburgh, PA) and treated with 30 μl of nutrient solution using a micropipette. Control baits were treated with 30 μl of distilled water. Treated paper baits and cover slips were placed in covered Petri dishes and allowed to air dry. The covered Petri dishes were stored unopened in sealed plastic storage containers (15cm x 12cm x 30cm; Newell Rubbermaid Inc., Freeport, IL), at room temperature, until needed for bioassay.

Table 6.1. Nutrients evaluated in termite feeding bioassays.

Nutrients	Source	Grams / Mole
Sugars		
Arbutin	ICN Biomedicals, Inc., Aurora, OH	272
d (-) Fructose	Sigma Chemical Co., St. Louis, MO	180
d (-) Galactose	Difco Laboratories, Detroit, MI	180
d (+) Glucose	Sigma Chemical Co., St. Louis, MO	180
d (+) Raffinose	Sigma Chemical Co., St. Louis, MO	505
Sucrose	Sigma Chemical Co., St. Louis, MO	342
d (+) Trehalose	Sigma Chemical Co., St. Louis, MO	339
Xylose	Fisher Scientific, Fairlawn, NJ	150
Nitrogen Compounds		
l-Arginine	Sigma Chemical Co., St. Louis, MO	174
l-Aspartic Acid	Sigma Chemical Co., St. Louis, MO	133
l-Cysteine	Sigma Chemical Co., St. Louis, MO	121
l-Isoleucine	Sigma Chemical Co., St. Louis, MO	131
l-Lysine	Sigma Chemical Co., St. Louis, MO	183
l-Methionine	Sigma Chemical Co., St. Louis, MO	149
l-Phenylalanine	Sigma Chemical Co., St. Louis, MO	147
l-Proline	Sigma Chemical Co., St. Louis, MO	115
Uric Acid	Sigma Chemical Co., St. Louis, MO	168

Laboratory bioassay arenas*.* Bioassay arenas consisted of Petri dishes (100 mm x 15 mm; Fisher Scientific, Pittsburgh, PA) filled with a sand substrate. The sand (Playsand, Quikcrete™ Corporation, Atlanta, GA) was washed in tap water and dried in an oven for $\geq$ 48 hours at 270°C prior to use. The clean dry sand was then divided and weighed into 20 g units. Aliquots of sand were stored in plastic storage bags (16.5 cm x 8.25 cm; S.C. Johnson and Son Inc., Racine, WI). Distilled water (3 ml; 15 % by weight) was added to each bag of sand. The bag was resealed and kneaded by hand to mix sand and water evenly. Moistened sand was placed into the assay arenas and spread evenly over the bottom of the Petri dish.

Choice tests. Bioassay arenas used in the choice feeding tests contained two baits; one bait treated with a nutrient solution, and a control bait treated with distilled water (Fig. 6.1). The baits were positioned side by side in the center of the arena. Position of baits within the arena was alternated among the replicates.

No-choice tests. Bioassay arenas used in the no-choice feeding tests contained one bait that had been treated with a nutrient solution or one control bait treated with distilled water. Baits were positioned in the center of the arena (Fig. 6.3).

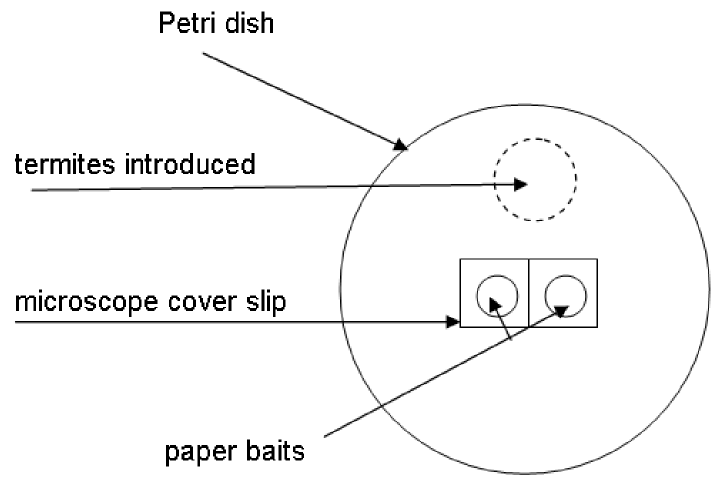

Figure 6.1. Design of the bioassay arena used in the choice bioassay containing one nutrient treated bait and one control bait.

Environmental chamber. An environmental chamber (EC) was used to exclude light and elevate humidity (>95% RH) in the bioassay arenas (Fig. 6.2). The EC consisted of a 113.7 L, snap-top plastic container (Cornerstone Products, Northglenn, CO) with a 6mm hole drilled in the base. Plastic tubing (5mm O.D.; New Age Industries, Inc. Southampton, PA) was inserted into the hole in the container and fixed in place using silicone glue (General Electric Sealants and Adhesives, Charlotte, NC). The free end of the tubing was fitted over the nipple of a Model 10 Whisper Air Pump (Tetra Corporation, Blacksburg, VA). A two-inch layer of pea gravel (Deco Pebbles, Quikcrete™ Corporation, Atlanta, GA) was placed into the bottom of the EC. Tap water was added to cover the gravel surface. The pump provided airflow (167 ml/min) over the water saturated gravel, resulting in chamber RH of approximately 95%.

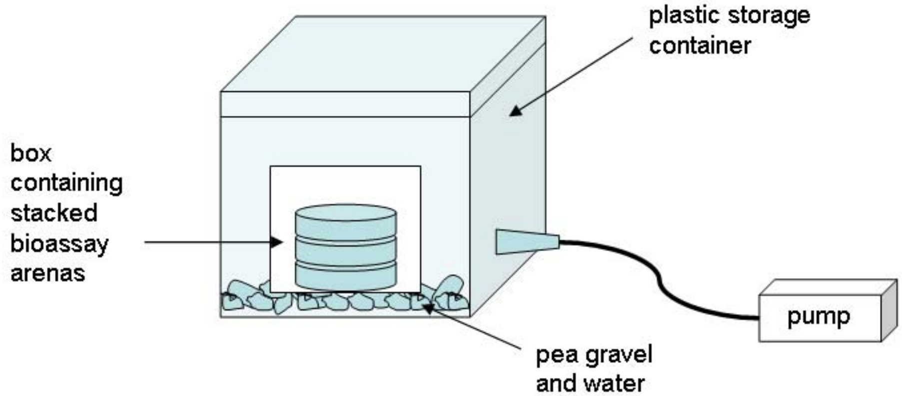

Figure 6.2. An environmental chamber (EC) was used to elevate humidity (95% RH) in the bioassay arenas.

Choice bioassay design. Prior to testing, populations of subterranean termites (50 workers) were removed from rearing containers with an aspirator and transferred into small plastic Petri dishes (5 x 15 mm). The lid was placed on each Petri dish and the termites were allowed to acclimate for 2 h. After the acclimation period, the lid of the Petri dish was removed and the dish was inverted releasing the termites in the bioassay arena adjacent to the paper baits. The bioassay arenas were placed into snap-top, plastic, storage containers (13 cm x 13 cm x 18 cm; Dollar Tree Corp., Chesapeake, VA). The storage containers were then placed into the environmental chamber (EC; Fig. 6.2). The termites were allowed to forage *ad libitum* for 96 h. After the foraging period, the storage containers were removed from the EC and consumption of the paper baits was recorded. A sheet of clear plastic marked with a 1 mm grid was placed directly over each bait and the area consumed was colored in with a fine-point, felt-tip pen. The number of colored squares on the grid was counted and the total area of paper bait consumed was calculated (mm^2). Replications of each choice bioassay were performed using termites from five different populations. Each bioassay had 13 replicates.

No-choice bioassay design. Nutrients that stimulated significantly higher (or lower) levels of consumption in the choice bioassays were evaluated further in no-choice tests. The purpose of the no-choice evaluation was to determine if those nutrient treated baits, that had significantly higher consumption in the choice tests, were simply preferred over the controls or were actually phagostimulants, causing the termites to eat more of the nutrient treated bait than they would of a control bait in a no choice test. Likewise, those nutrient treated baits that were consumed significantly less than the control bait in the choice tests were further evaluated to determine if they were just non-preferred, or actual feeding deterrents, causing the termites to eat less of the nutrient treated bait than they would of control bait. Preferred nutrient compounds were compared at concentration that resulted in the highest consumption observed, during the choice tests. Materials that inhibited feeding in the choice bioassay were assayed at the lowest concentration at which a significant reduction in feeding was observed. The design of the no-choice bioassay was the same as the choice bioassay except that a smaller population of 30 termites was used (Fig. 6.3). Each bioassay had 8 replicates.

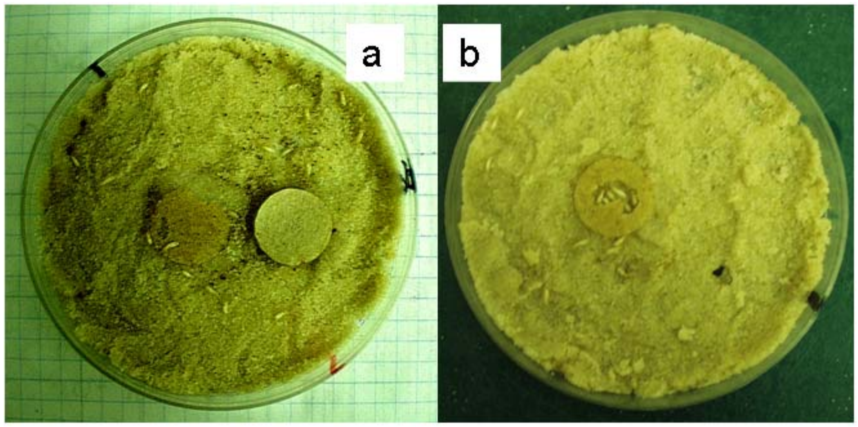

Figure 6.3. a. Choice bioassay arena b. No-choice bioassay arena

Statistical analysis. For the choice tests, the mean proportion of consumption, of each nutrient treated bait at each dilution, was compared with the mean consumption of control bait using χ^2 analyses (PROC GENMOD, SAS Institute, 1999). In the no-choice tests, mean consumption for the treated baits and the control bait was compared using ANOVA (PROC GLM, SAS Institute, 1999). For all tests values of P values $\leq$ 0.05 indicated significance.

Results

Choice bioassay. The influence of nutrient additives on subterranean termite bait consumption varied considerably across the nutrients tested. While several of the sugars enhanced feeding, others deterred feeding or had no effect, depending on the concentration. The nitrogen compounds generally deterred feeding at the highest concentrations and had no significant effect at the two lower concentrations (Table 6.2).

Several of the sugars elicited preferential feeding on paper baits in the choice bioassay. Termites fed preferentially on baits treated with all three concentrations of galactose. Baits treated with glucose, sucrose, and trehalose were fed on preferentially at the highest concentration (0.1 M) but were not significantly different from the controls at the 0.01 and 0.001 concentrations. Fructose and raffinose treated baits were fed on preferentially at the 0.01 M concentration only. Xylose treated baits were not significantly different from the controls for any of the concentrations tested. Consumption of baits treated with arbutin was very low and arbutin treated baits were consumed significantly less than the controls at all three concentrations.

None of the nitrogen compounds except uric acid at the lowest (0.001 M) concentration significantly enhanced termite feeding. Aspartic acid, isoleucine, arginine and phenylalanine significantly reduced bait consumption at the highest concentration (0.1 M), but were not significantly different from the control baits at the two lower concentrations. Consumption of proline treated baits was significantly lower than the control baits at both the 0.01 M and 0.1 M concentrations but was not different from the controls at the 0.001 M concentration. Cysteine and methionine treated baits did not significantly influence consumption at any of the concentrations tested. Termite mortality was not measured in these bioassays; however, mortality was so high in the lysine bioassays (all concentrations) that no consumption data could be collected.

Table 6.2. Proportion of nutrient treated bait consumed (mm^2) compared to proportion of control bait consumed in choice bioassay.

Nutrients	**Percent consumption (mm^2) $\pm$ SEM of baits at treated with specific nutrient concentrations**		
	0.001 M	**0.01 M**	**0.1 M**
Sugars			
Arbutin	0 ###	12 $\pm$ 6 ###	3 $\pm$ 2 ###
d (-) Fructose	50 $\pm$ 17	76 $\pm$ 18***	46 $\pm$ 14
d (-) Galactose	71 $\pm$ 20***	83 $\pm$ 21***	76 $\pm$ 25***
d (-) Glucose	56 $\pm$ 15	59 $\pm$ 14	72 $\pm$ 11***
d (-) Raffinose	45 $\pm$ 11	68 $\pm$ 13**	69 $\pm$ 14
Sucrose	53 $\pm$ 11	39 $\pm$ 11	69 $\pm$ 16
Trehalose	46 $\pm$ 2	56 $\pm$ 17	84 $\pm$ 21***
Xylose	39 $\pm$ 13	53 $\pm$ 17	56 $\pm$ 12
Nitrogen Compounds			
l-Arginine	16 $\pm$ 6	12 $\pm$ 9	8 $\pm$ 16###
l-Aspartic acid	40 $\pm$ 12	40 $\pm$ 12	24 $\pm$ 7###
l-Cysteine	50 $\pm$ 16	50 $\pm$ 16	59 $\pm$ 12
l-Isoleucine	46 $\pm$ 16	49 $\pm$ 18	1 $\pm$ 5###
l-Methionine	48 $\pm$ 25	38 $\pm$ 21	62 $\pm$ 29
l-Phenylalanine	46 $\pm$ 13	28 $\pm$ 10	5 $\pm$ 3###
l-Proline	40 $\pm$ 12	5 $\pm$ 6###	6 $\pm$ 4###
Uric Acid	64 $\pm$ 24***	---	---

χ^2 Tests (PROC GENMOD; SAS Institute, 1999); ** Consumption significantly greater than controls ($\underline{P}$ = 0.01); *** Consumption significantly greater than controls ($\underline{P}$ = 0.001); $^{\#\#\#}$ Consumption significantly less than controls ($\underline{P}$ = 0.001).

No-choice bioassay. No-choice evaluations of the sugar treated baits indicated that none of the sugars tested were true phagostimulants because consumption of the treated baits was not significantly greater than the control baits (Fig. 6.4). Similarly, no-choice evaluations of the nitrogen compounds indicated that none of the compounds were true feeding deterrents because consumption of the nutrients treated baits was not significantly different from the controls for any of the compounds tested (Fig. 6.5).

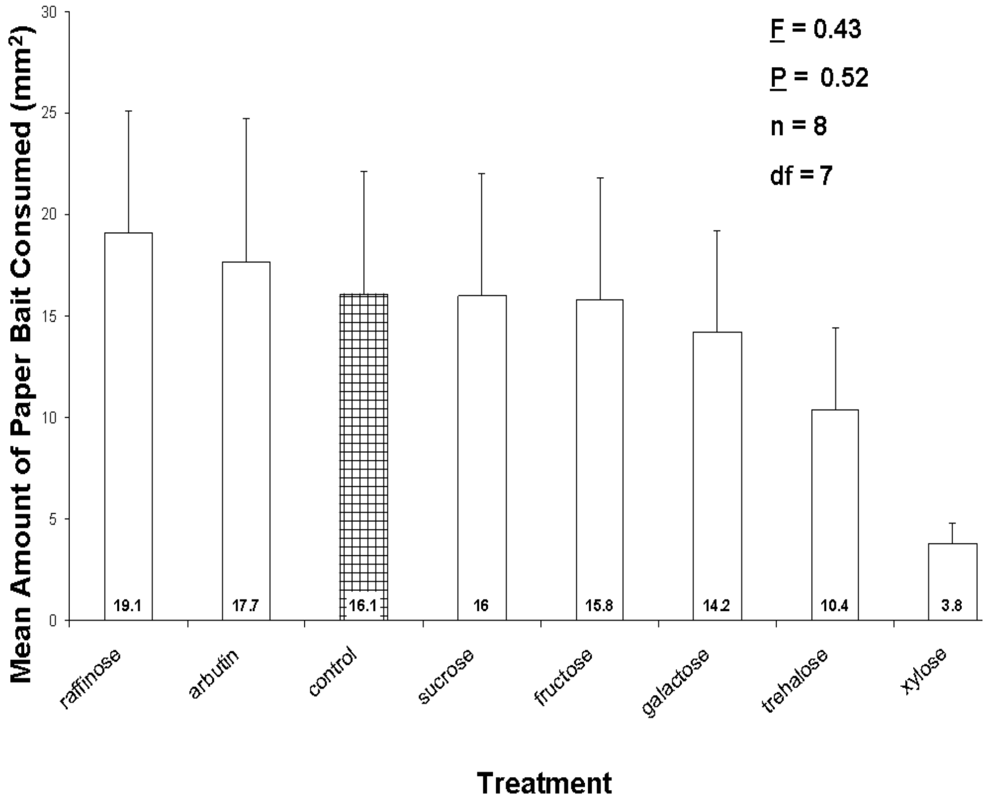

Figure 6.4. Comparison of subterranean termite consumption of baits (mm^2) treated with sugars in the no-choice bioassay. ANOVA (PROC GLM; SAS Institute, 1999.) Error bars indicate SEM.

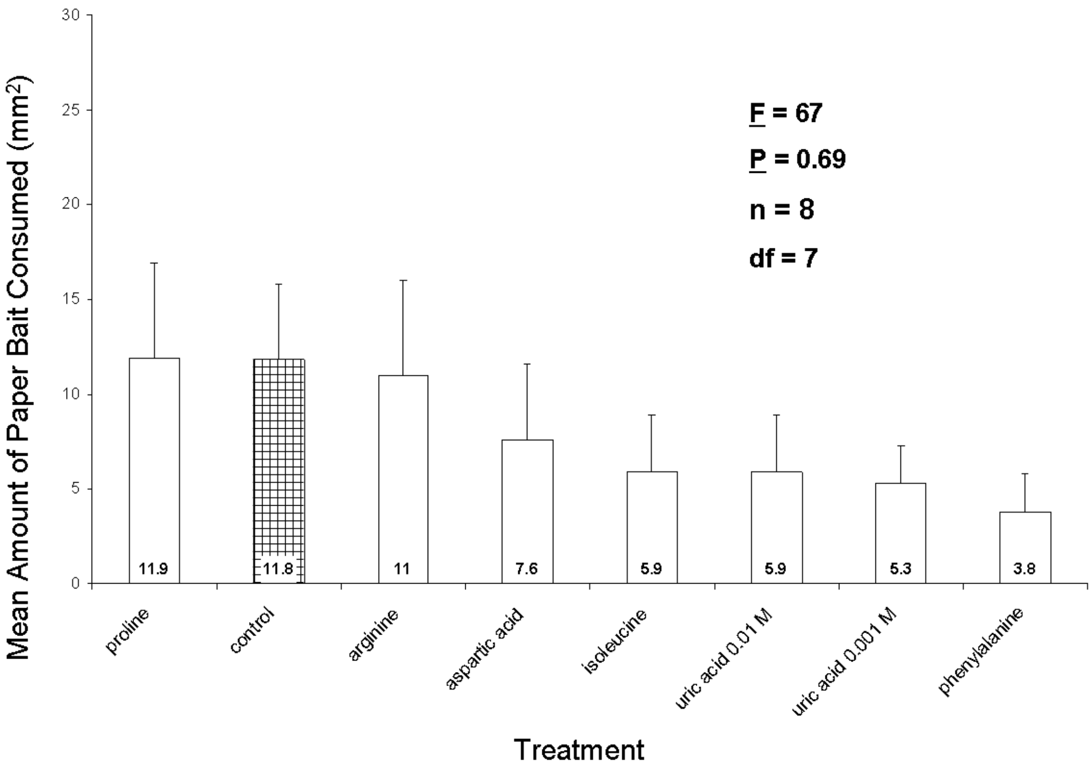

Figure 6.5. Comparison of subterranean termite consumption of baits (mm^2) treated with nitrogen compounds in the no-choice bioassay. ANOVA (PROC GLM; SAS Institute, 1999.) Error bars indicate SEM.

Discussion

Several of the results in this study support those of Waller and Curtis (2003) who also evaluated the consumption of paper baits treated with 3 sugars (glucose, sucrose and xylose at 1% and 3% concentrations) in choice arena tests. Like Waller and Curtis (2003), consumption of baits treated with the highest concentration of glucose (0.1 M or 2% and 3% concentrations, respectively) was significantly preferred by subterranean termites in choice evaluations. These results also concurred with those of Reinhard and Kaib

(2001) who determined that glucose acted as feeding stimulants for *R. santonensis* at concentrations of 0.01 M or greater.

Waller and Curtis (2003) had also reported that baits treated with 1% and 3% concentrations of xylose and sucrose were consumed preferentially over control baits. The results reported in this study did not indicate that xylose or sucrose enhanced feeding at any of the concentrations tested. A possible reason for this difference was that the Waller and Curtis (2003) study was conducted with 100 termites over a period of 5 weeks. This bioassay used half as many termites and consumption was measured after only 4 d. It is reasonable to suggest that xylose and sucrose treated baits may have had an influence on termite consumption but that the effects on termite feeding may have been more subtle than those of other sugars. If the termites had been given a longer feeding period, small differences in feeding behavior may have become more obvious.

In addition to those sugars identified by Waller and Curtis (2003), several additional sugars enhanced termite feeding in the choice tests. The most notable was galactose, which was fed on preferentially at all three concentrations. The preference for galactose might have been due to the fact that galactose is a natural component of wood, but occurs only at low concentrations (< 3%) in most wood species (Kaar and Brink, 1991; Kartal and Lebow, 2000; Tucker et al., 2001). Thus, it is possible that galactose stimulated termite feeding because it is a nutritional requirement of *Reticulitermes spp.* that is scarce in the termites' natural diet. Trehalose also elicited preferential feeding at the highest concentration 0.1 M (~3%). Interestingly, fructose and raffinose elicited preferential feeding by the termites but only at the mid-range concentration of 0.01 M (0.2% and 0.5% respectively). Because these sugars were not preferred at the higher concentrations additional testing would be necessary to determine if the feeding preferences were really concentration dependent or if higher concentrations would also enhance feeding if the termites were tested for a longer period.

Reinhard and Kaib (2001) reported that arbutin was a strong feeding stimulant for *R. santonensis* at a 0.1 M dilution and a mild stimulant at 0.01 M dilution. However, in the choice bioassay described here, arbutin significantly reduced feeding by *Reticulitermes spp.* at all levels tested. It is possible that this discrepancy was due to differences in the experimental design. While the choice bioassay measured the amount of paper consumed by termite workers over a period of 96 h, Reinhard and Kaib (2001) measured feeding preference by counting the number of termites aggregating (feeding inferred) on the bait over a one-hour period. The use of aggregation as an indicator of feeding preference may not be a reliable method of evaluation. Mishra (1992) found that the initial orientation and supposed preference of *Neotermes bosei* Snyder for chemically treated paper baits was not always followed by bait consumption. Baits initially evaluated as attractive were sometimes later abandoned by the termites. Likewise, baits that were initially ignored during the first 30 minutes of a bioassay were sometimes preferentially consumed after 24 h of *ad libitum* foraging (Mishra 1992). Therefore, Reinhard and Kaib's (2001) results may have only reflected an initial attraction of termites to arbutin treated baits, while our tests measured actual consumption.

In choice tests evaluating the nitrogenous compounds, only uric acid at the 0.001M concentration, elicited preferential feeding. Subterranean termites are known to regularly consume nitrogen in the form of uric acid when they consume the bodies of nestmates (Hingate, 1941; Potroikus and Breznak, 1981). Because of the very low nitrogen content of wood (> 0.05%; Hingate, 1940), cannibalism and necrophagy are important adaptive strategies that conserve nitrogen within the subterranean termite colony (Hingate, 1941; Potrikus and Breznak, 1981). Uric acid accounts for approximately 3% of the total termite body weight and up to 23% of the gut weight (Potroikus and Breznak, 1981). Because uric acid is such an important nutrient for subterranean termites, it was fully expected that the uric acid treated baits would be significantly preferred at all concentrations. However, because of the insolubility of uric acid, it was not possible to formulate solutions at the two higher concentrations, 0.1M and 0.1 M. Suspensions were prepared at these concentrations. When these suspensions were applied to the paper baits, crystals of uric acid were observed forming on the bait surface as the baits dried. Initially attempts were made to bioassay these baits but consumption was very low. The lack of consumption might have been interpreted as feeding inhibition; however, it was not possible to determine if the termites were consuming the crystals of uric acid off of the paper instead of consuming the paper itself. In addition, the precipitation was found to have greatly reduced the concentration of uric acid applied to the bait matrix. Not knowing the concentration of uric acid on the bait matrix confounded the consumption results. For these reasons, the higher concentrations of uric acid were eliminated from the analysis and only the lowest concentration was used.

None of the other nitrogenous compounds evaluated in the choice tests were fed on preferentially. However, several of the amino acids (aspartic acid, isoleucine, phenylalanine, and proline) reduced bait consumption at the highest concentrations. These results were somewhat unexpected because an earlier study (Chen and Henderson 1996) evaluating nutrient consumption by *C. formosanus* found that these same amino acids did not decrease bait consumption. This difference in feeding behavior between *C. formosanus* and *Reticulitermes spp*. may be due to the fact that *C. formosanus* consumes both living and dead wood (Chen and Henderson, 1996). Living plants contain free amino acids that are used for metabolic processes (Yamashita, 1990; Nordin et al., 2001). North American *Reticulitermes spp*. consume dead wood almost exclusively. As dead wood begins to decay, changes in the chemical structure occur. Amino acids are frequently leached from the wood over time or combined into different compounds by decay fungi (Kozlowski and Pallardy, 1997).

The termite mortality observed in the lysine choice tests was completely unexpected. In previous studies, lysine was identified as a phagostimulant for both *R. santonensis* (Reinhard and Kaib, 2001) and *C. formosanus* (Chen and Henderson, 1996). Yet, mortality in the choice tests was so high it was impossible to record any consumption data. I did not quantify termite mortality but mortality was observed in all replications (evaluated on two different days), using termites from 5 different colonies. It is possible that the lysine sample used in this bioassay was contaminated and additional bioassays should be performed using a different nutrient sample before any inferences are made about the effects of lysine on termite bait consumption.

The purpose of the no-choice bioassays was to further identify those nutrients that were preferred or not preferred in the choice tests, as true phagostimulants or feeding deterrents. The fact that consumption of bait treated with either the sugars or nitrogenous compounds was not significantly different from the controls indicated that none of the compounds tested could be considered phagostimulants or feeding determents. The no-choice bioassays determined that all of the nutrient treated baits were palatable. Even consumption of the arbutin treated baits was not significantly different from that of the controls in the no-choice tests. This finding confirmed that subterranean termites will feed on food resources that are available, even if they would prefer to eat something else (Wood 1978). The fact that none of the preferred baits from the choice bioassays elicited significantly more consumption than the control baits indicated that in the presence of any palatable food resource, a given number of termites can only consume a certain amount of bait in a specified period of time.

The data presented in this study suggest that sugars and nitrogenous compounds can influence subterranean termite bait consumption even though they are not true phagostimulants or feeding determents. Formulations of galactose, glucose, trehalose, raffinose and uric acid have the potential to enhance termite consumption of baits in the presence of competing food resources. However, before these nutrients can be recommended for inclusion in commercial baiting systems their susceptibility to biodegradation needs to be considered.

Microbial degradation of supplementary nutrients for termite baits was not evaluated in this study, nor has this topic been given consideration by other researchers evaluating termite feeding preferences (Chen and Henderson 1996; Waller and Curtis 2003). However, microorganisms can readily consume and metabolize nutrients applied to a cellulose matrix (paper). Consumption of these nutrients by microorganisms could rapidly decrease the nutrient concentration within the bait matrix. Microorganisms also produce waste materials that could contaminate the bait and ultimately inhibit termite feeding. Nutrient degradation by microorganisms can be prevented in laboratory studies if the baits are prepared and stored under sterile conditions prior to bioassay. However, once the baits are put into the bioassay arenas (or in the field) the nutrients in the bait matrix are no longer protected. The effects of microbial degradation on the concentration of nutrients evaluated in this bioassay are unknown. However, these effects need to be thoroughly understood before any of these nutrients could be recommended for inclusion in a commercial termite baiting system.

Chapter VII: SUMMARY

The purpose of this series of studies had two goals. First, to identify means of reducing the time required for subterranean termites to discover commercial bait stations. Second, to identify strategies for reducing the likelihood that subterranean termites would desert a commercial bait station once it had been discovered.

Only one of the strategies investigated in this group of experiments appears to hold some promise for reducing the time required for subterranean termites to discover commercial bait stations. The bioassay to determine the effect of thermal shadows on termite aggregation, described in Chapter V, clearly demonstrated that *Reticulitermes* spp. workers aggregate in cool thermal shadows (areas of soil that are significantly cooler than the surrounding substrate). A thermal shadow around a commercial bait station might act as an attractant, by creating a temperature gradient in the substrate, which could be detected by termites some distance from the bait. The use of thermal shadows as a termite ‘attractant’ is certainly worthy of further investigation. The possibility that substrate temperature might act as a subterranean termite attractant is especially exciting because several studies have demonstrated that termites cannot detect palatable foods through the substrate, even over very short distances (Cornelius and Osbrink, 2001; Puche and Su, 2001b; Campora and Grace 2001). Thus it seems unlikely that an effective chemical attractant will ever be found. Many commercial bait stations already have a collar lying flat on the soil surface that is broader than the bait receptacle. If a 12-inch collar was coated with a reflective material or a plastic designed to function as a heat sink, it would be possible to create a thermal shadow around the bait receptacle. Manipulating substrate temperature to create cool thermal shadows around bait stations might be a way to increase the probability of bait discovery and thus decrease average time to commercial bait discovery in the field.

We found that it was impractical to direct subterranean termites toward in-ground baits, using physical guidelines, under field conditions. Connecting baits with physical guidelines increased the likelihood of bait discovery in the laboratory. In the laboratory bioassay arenas, the guidelines were a unique artifact in an otherwise homogenous environment. The termites' centered their activity around the guidelines because there were no other orientation cues. Under field conditions, foraging termites encounter many environmental influences, including variations in available moisture, thermal shadows, predators and potential food items. Guideline following is one foraging strategy of subterranean termites, but it is not the only strategy termites use to locate food. It seems likely that there is a hierarchy of stimuli that affect the termites' foraging and feeding activity. The influence of a physical guideline may be less important than the stimulus of a significant thermal shadow or a chemical cue. When we attempted to direct termite movement through the soil with guidelines under field conditions, no response was observed.

One finding of the bioassay to determine the influence of physical guidelines on subterranean termite bait consumption and tunneling behavior may hold some promise for creating bait stations that are less likely to be deserted. In the laboratory, subterranean termites built significantly longer tunnels adjacent to guidelines containing cellulose as compared to plastic guidelines. A novel class of building materials, wood thermoplastic composites (WTC), elicited tunneling activity similar to white pine. In spite of the fact that termites are unable to consume significant amounts of WTC, they explored these materials extensively, in they same way they explore food. Wood thermoplastic composites are more resistant to insect attack and fungi than wood. Wood thermoplastic composites can be molded in the same way as a plastic. If termite foragers would continue to investigate a WTC bait station as if it were a food resource, after the bait matrix was depleted, subterranean termites would be less likely to desert WTC bait stations, in favor of another food resource. This idea is worthy of further investigation.

In our bioassay to compare sugars and nitrogen compounds as nutrient compounds for *Reticulitermes* spp.*,* we determined that *Reticulitermes* spp. consumed significantly more paper bait treated with uric acid and several sugars than plain paper baits. Treating bait matrix with preferred nutrients like galactose or uric acid might help to prevent the desertion of bait stations in the field in favor of other food resources by presenting a bait matrix that would be preferred to competing food resources.

It may be possible to decrease the time required for foraging subterranean termites to discover commercial bait stations in the field by surrounding the station with a cool thermal shadow. This could be accomplished by providing the station with a collar on the soil surface composed of a reflective material or a material that acts as a heat sink.

Subterranean termites are less likely to desert highly palatable food resources so treating bait matrices with nutrients known to be palatable could decrease the likelihood that commercial bait stations would be deserted. In addition it might increase the amount of bait consumed and thus the amount of toxicant ingested. We identified several substances that were highly palatable to the termite populations tested including galactose and low concentrations of uric acid.

In addition, termites were shown to build significantly longer galleries adjacent materials containing cellulose, possibly in an effort to analyze the food value of the material. Plastics do not trigger this increased tunneling response. Thus, it is highly unlikely that plastic bait stations are objects of interest when foraging subterranean termites in the field encounter them. Indeed, there is even evidence that some plastics are repellant to subterranean termites. We identified a novel class of building materials, the wood thermoplastic composites which, while not of significant nutritional value to subterranean termites, stimulated gallery building as effectively as white pine. Bait stations fabricated of a wood thermoplastic composite might be less likely to be abandoned than plastic bait stations, since they trigger this search activity in termite foragers. This idea is worthy of further investigation.

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