SUBTERRANEAN TERMITES OF THE OKLAHOMA TALLGRASS PRAIRIE PRESERVE CROSS TIMBERS

By

MATTHEW PAIGE SMITH

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CROSS TIMBERS

Thesis Approved:

Bradford M. Kard
Thesis Adviser

Udaya DeSilva

Jack W. Dillwith

Mark E. Payton

A. Gordon Emslie
Dean of the Graduate College

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CHAPTER I

INTRODUCTION

Termites are valuable components of the ecosystem, recycling cellulose material and influencing soil composition and structure. The majority of structures in the United States of America (USA) are wooden and susceptible to termite attack. In the USA, termites are responsible for $1 billion to $11 billion in annual expenditures for termite preventive treatments and repairs of structural damage.

An increased knowledge of the basic biology and behavior of *Reticulitermes* sp. (Isoptera: Rhinotermitidae) is important to better understand how termites affect their environment in both a natural settings and urban landscapes. Termites that are responsible for the majority of damage to wooden structures in the USA are subterranean in nature, making it difficult to gather data on the size and distribution of their colonies. Several studies have been completed that investigated termite behavior in Ontario, Canada, and throughout the USA. In addition to providing insight into termite biology, one purpose of these studies was to gather information to help effectively manage termite infestations in structures. Currently, there are two primary methodologies used by pest management professionals to prevent and treat termite infestations. The first methodology is to treat the soil around and below a structure with termiticide to create a continuous chemical barrier. The second methodology is to emplace baiting stations

around a structure. For termite baiting systems to be effective it is imperative that foraging behavior of termites be understood.

In Oklahoma, the population density of subterranean termites in soil ranges from relatively moderate to heavy across the state, except for the counties located in the extreme southeast where densities are considered 'very heavy'. To date, four studies documenting distribution and behavior of termites in Oklahoma have been completed for limited geographic areas. The study that is the subject of this thesis was conducted on the Nature Conservancy's Tallgrass Prairie Preserve (TGPP) Cross Timbers, 16km north of Pawhuska, Oklahoma, in Osage County. "The cross timbers is a mosaic of xeric oak woodlands with patches of savanna and prairie openings, covering approximately 4.8 million ha primarily in central Oklahoma and northern Texas" (Clark and Hallgren 2003). The study focuses on a Cross Timbers area where prescribed burns are conducted, and a Cross Timbers area excluded from burning.

It is critical that termites be identified to species when analyzing and subsequently reporting behavior. Morphological dichotomous keys for identification of alates and soldiers of *Reticulitermes* sp. have existed since 1920, but differentiation between certain similar species has been tenuous. The development of improved computerized microscope ocular equipment and associated software has advanced our ability to accurately measure and identify termites. However, due to overlapping measurements in morphological characteristics between *R. flavipes* and *R. virginicus*, the best ocular equipment still does not always afford positive separation and identification of these two species. Alates may not be present in a colony until it is five-to-seven years old, and then may only be present once or twice a year. Soldiers are always present in mature

Reticulitermes colonies, but because they are not evenly distributed throughout the colony they may not be always present when a sample of termites is collected. The most abundant caste in termites and thus the most commonly collected is the worker. Whereas several keys exist for the identification of alates and soldiers, due to the similar appearance of workers of different species no morphological keys utilizing workers can be developed. Thus, the worker caste cannot be used for morphological identification. However, advancement of molecular techniques has made positive identification possible as termites may be identified genetically, regardless of caste.

The overall goal of this research is to increase knowledge of *Reticulitermes* foraging behavior and colony characteristics. Additionally, studies within two differing Cross Timbers habitats, when added to previous studies conducted on the tallgrass area will provide additional information on termite biology and behavior on the TGPP.

Objectives

1. Morphologically identify termites, and verify identifications with molecular techniques using a region of the mtDNA 16S rRNA gene.
2. Delineate five termite colony foraging territories.
3. Estimate numbers of foraging termites within each colony.
4. Compare estimates of foraging territories in two different areas of the Cross Timbers.
5. Determine soldier percentage within each colony.

CHAPTER II

REVIEW OF LITERATURE

General

Termites are eusocial insects and the lone members of the insect order Isoptera. Isoptera is derived from the Greek words “iso” and “ptera”, “equal wings”, referring to the equal length and shape of the fore and hind wings of the insect. This order is divided into seven families, Hodotermitidae, Kalotermitidae, Mastotermitidae, Rhinotermitidae, Serritermitidae, Termitidae, and Termopsidae, that are currently divided into 281 genera containing more than 2,700 described species (Jones 2000). A common way of classifying termites is to refer to their preferred habitats, i.e., dampwood, drywood, or subterranean. These terms are general classifications based on habits and nesting locations.

Termites have a caste system. For subterranean termites, the monogamous king and queen are responsible for reproduction, and as the colony grows larger supplementary reproductives may develop. For almost all species, a soldier caste exists for protection of the colony, whereas workers maintain the colony, feed their nestmates, and care for the brood. Termites are a valuable part of their ecosystems (Wood and Johnson 1986). They are the dominant invertebrates found in tropical habitats (Wood and Sands 1978,

Eggleton et al. 1996), and have a major effect on soil structure and composition, nutrient cycling,and plant growth (Lee and Wood 1971, Wood and Sands 1978). Termites not only occupy a specific niche, but they also modify their surroundings and nest areas in such a way that it meets their habitat requirements (Bouillon 1969). Termites serve an important role throughout much of the United States and the world in the turnover of cellulose-containing plant material (La Fage and Nutting 1978). In temperate areas of the world, termites have been reported to consume 16.6% of the wood, twigs and sticks that fall annually (Lee and Wood 1971). On the Guinea savanna of Nigeria, 23% of leaf litter was removed by termites (Collins 1981).

Although they serve important ecological purposes, termites are better known for the negative impact they have on wooden structures. The Romans referred to termites as "Termes", which means "woodworm" (Potter 2004). Termite damage is not limited to only the wood in a structure, but may extend to drywall, stucco, and wiring insulation. The estimated annual cost in the USA for termite preventive and remedial treatments, and repair of structural damage, is estimated to range from over $3 billion to $11 billion. An estimated $3 billion to $5 billion of this cost is attributed to structural damage (Thorne 1998, Jones 2000, Su 2002, Virginia Pest Management Association 2006). Termites are found in every state in the USA except Alaska (Suiter et al. 2002). In the USA there are five families of termites: Hodotermitidae, Kalotermitidae, Rhinotermitidae, Termitidae, and Termopsidae (Weesner 1965, Thorne et al. 1993). Of the approximately 50 species of termites in the USA, 30 species are economically important (Su and Scheffrahn 1990). The species responsible for most of the damage to wooden structures in the USA is the Eastern subterranean termite, *Reticulitermes flavipes* (Kollar) (Potter 2004), especially

east of the Rocky Mountains. Two termite species of major economic importance west of the Rocky Mountains are the Western subterranean termite, *R. hesperus* Banks and the Desert subterranean termite, *Heterotermes aureus* (Baker and Bellamy 2006). Another species of major economic importance in the USA is the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. Although this species has only been found in 11 states (Su and Tamashiro 1987, Potter 2004), it is responsible for $1 billion in preventative treatments, remedial control, and damage and repair costs, annually (Lax and Osbrink 2003). Other USA species of economic importance are the Dark Southeastern subterranean termite, *R. virginicus* Banks, the Light Southern subterranean termite, *R. hageni* Banks, and the Arid Land subterranean termite, *R. tibialis* Banks.

Termites endemic to Oklahoma are *R. flavipes*, *R. virginicus*, *R. hageni*, *R. tibialis* and *Gnathamitermes tubiformans* (Brown et al. 2008, Smith 2008). In Oklahoma, the density of subterranean termites ranges from moderate-to-heavy across the state except for the extreme southeast counties where densities are greatest (Jones 2000, Suiter et al. 2002).

Methods of Termite Management

Currently, five methods are employed to either prevent or treat attacks by termites on wooden structures: chemical barrier treatments to soil, physical barriers, wood preservatives, construction techniques and materials to build-out termites, and baiting systems. Today there is a large emphasis on how each of these types of treatments affects the environment. These treatments have been modified through the years to reduce their environmental impact, and this effort continues today. Treatment choices are

not based solely on how well they protect against termite infestation, but also on how a particular treatment may impact the environment.

Chemical Barrier Treatments

Chemical barrier treatments have been used for approximately 60 years. A liquid or granular chemical barrier is established in the soil immediately around and under a structure to either repel or kill termites. Pre-construction 'horizontal' insecticide treatments to soil are achieved when a termiticide is sprayed on the soil surface over the areas where a concrete foundation will be poured. Vertical trench chemical barriers are also established along the interior and exterior stem walls. Hollow concrete-block walls and crawl spaces are treated with termiticide both before and after construction. The purpose of these termiticide treatments is to provide a chemical barrier in the soil (and inside exterior hollow-block walls) that is continuous on the structure periphery, within concrete blocks, and immediately underneath the foundation. Failure to control termites usually occurs due to gaps in the chemical barrier (Mampe and Bret 1992, Forschler 1994, Kuriachan and Gold 1998).

Early 20th century insecticides used for termite control were cyclodienes, a subclass of organochlorines. Cyclodienes are GABA-gated chloride channel antagonists that inhibit GABA from signaling the release of chlorine ions. This causes a repeated synaptic discharge that eventually kills the insect (Ware 2000). Chlordane was introduced into use in 1948 and used extensively until removed from the market in 1988. It is estimated that over 30 million homes in the USA were treated with chlordane, and that it has been shown to be effective for 35 years or more (Kard et al. 1989). Organophoshate and pyrethroid insecticides have also been used to control termites.

Organophosphates function as acetylcholinesterase inhibitors. This inhibition results in neuromuscular paralysis and eventual death because acetylcholine is not removed from the post-synaptic receptor gate (Ware 2000). Pyrethroids are sodium channel modulators that prolong the sodium ion current (Ware 2000).

Insecticides currently used to control termites are designed to be less toxic to mammals and to have less residual effect in the soil. Five years of residual activity is now common, compared with the 30 years or more of residual activity for the early pesticides. Pyrethroids and pyrazoles are two of the chemical classes widely used as termiticides today. An example of each is Talstar® (FMC Corp., Philadelphia, PA) that contains the pyrethroid bifenthrin, and Termidor® (BASF, Mt. Olive, NJ) that contains the pyrazole fipronil. Fipronil is a GABA-gated chloride channel antagonist (Ware 2000). Osbrink et al. (2001a) reported substantial inter-colony and intra-colony differences in the susceptibility in *R. flavipes* and *C. formosanus* to insecticides. They also suggest that treatment failure may not be due to incomplete or improper application of termiticide, but may be due to decreased susceptibility of the termites. In contrast, Valles and Woodson (2002) found that *C. formosanus* was uniformly susceptible to termiticides, but may possess the ability to become tolerant to termiticides. Their social structure, in particular the reproductive dependence on a single primary queen, may retard this tolerance. Another potential negative to the use of liquid termiticide barrier treatments is that they prevent termites from infesting the building but do not eliminate a colony. The colony may continue to thrive and become large enough that alates are produced to start new colonies (Su 2005).

Physical Barriers

Chemical treatments to soil utilize insecticides to create a toxic barrier to termites, whereas physical barriers exclude termite activity by utilizing mechanical barriers. Sized sand particles, stainless steel mesh, or insecticidal vapor barrier sheeting may be used to cover the soil surface beneath, or incorporated into, a structure. Metal termite shields also stop termites from entering a structure through the stem wall. Physical barriers may cost as much as 25% more in initial cost compared with chemical barriers, but may last at least ten times longer (Rawat 2002).

Particulate barriers may consist of sized particles of basalt, granite, limestone, or silica sand. It has been shown that when these sands are of a specific size, termites cannot tunnel through them (Ebeling and Pence 1957, Tamashiro et al. 1987, Smith and Rust 1990, Su and Scheffrahn 1992, Myles 1997). Particulate barriers are used primarily in Hawaii, where a 10.2cm (4in)-deep layer of Basaltic Termite Barrier® (Ameron, Oahu, HI) is installed under and around a structure to protect from attacks by *C. formosanus*, which causes $100 million in yearly damage in the Islands (Yates et al. 1999, Yates et al. 2000). Use of particulate barriers elsewhere in the USA is limited due to the difficulty and cost of installation, and because the efficacy of particulate barriers are compromised if the barrier is disturbed due to landscaping, remodeling, or rodent activity.

The oldest form of physical barriers for termites are metal termite shields (Potter 2004). These shields are installed as a continuous metal sheet on top of stem walls or foundation piers, forcing the termites to tunnel over the shield and away from the stem wall or foundation surface to reach the structure. Termite shields do not prevent termite activity but force termites to build their mud foraging tubes in visible areas. Once mud

tubes are discovered the building receives a termiticide treatment. Metal shields must be installed properly or their function will be of little value. Upon inspection of 310 houses, Hamilton and Cobb (1964) found so many problems with incorrect installation and subsequent damage, that they deemed that metal shields were ineffective.

Stainless steel mesh or plastic sheeting impregnated with insecticide is used as a pre-construction method to physically block termite infestation. Termi-Mesh® (Termi-Mesh, Perth, Australia) is a stainless steel mesh placed on prepared foundation soil fill before the concrete foundation is poured. The mesh aperture is small enough to exclude termites. It was developed in Australia and is shown to be an effective termite exclusion barrier in tests there and in USA (Su and Scheffrahn 1992, Lenz and Runko 1994, Grace et al. 1996, Ewart 2001, Kard 2003). According to Takahashi and Yoshimura (2002), Japan is using the product to protect structures against two destructive species of termites. Termi-Mesh is placed in a continuous layer on the soil area prepared for construction of a building's foundation. Before the concrete is poured all pipe and utility penetrations are wrapped with Termi-Mesh 'boots' to prevent a gap that would enable termite entrance. Lenz and Runko (1994) report that Termi-Mesh protection should outlast the building. This type of system contains no chemicals, thus the environment is not negatively impacted due to its utilization to prevent termite infestation.

Plastic sheeting impregnated with insecticide serves the same purpose, and is installed in a similar manner as Termi-Mesh. One added advantage to this method is that the sheeting also functions as a moisture barrier. Early studies of polyethylene sheets impregnated with lambda-cyhalothrin provided protection of greater than five years (Su et al. 2004a). Results of these tests were used to develop the Impasse® System

(Syngenta, Wilmington, DE). Impasse polyethylene sheets are layered, incorporating a lambda-cyhalothrin impregnated center layer. If tunneling termites penetrate the outer layers of Impasse sheeting in contact with the ground, they are repelled by the insecticide-treated center layer. The insecticide is impregnated into a matrix, thus protecting anyone working with the sheeting from insecticide exposure. This also minimizes the exposure of the soil to insecticide and protects the environment from contamination. Syngenta discontinued the manufacturing of Impasse in 2008, and no other manufactures in the USA are currently offering this type of product.

Wood Preservatives

Preservative treatment of wood used in construction is an effective tool in the prevention of termite infestations. Borate compounds have been proven to cause termite mortality (Maistrello et al. 2001). Although many types of wood preservatives are currently used, the water soluble chemical most commonly used is disodium octaborate tetrahydrate (DOT). Products using DOT are Bora-Care® (Nisus Corp., Rockford, TN), Tim-Bor® (Nisus Corp., Rockford, TN), Board Defense® (InCide Technologies, Phoenix, AZ) and Borrada D™ (Control Solutions Inc., Pasadena, TX). Methods of treating lumber are dipping green lumber in a DOT hot bath, pressure-treating lumber with DOT, and spraying DOT on the framing of a structure. Whereas Australia and Europe have used borate-treated wood for greater than 50 years (Murphy 1990), boron treatment of wood in the USA began in 1982, and studies on the use of borate-treated wood in the USA are more recent (Williams 1984, Williams and Mauldin 1985, 1986). Studies on boards fully penetrated with DOT show the utility of borate containing products in the reduction of termite damage (Grace and Yamamoto 1994, Mauldin and Kard 1996). The

treatment for structures recommended by Nisus (2006) for Bora-Care is the partial spraying of the foundation, sill plates, and lower 61.0cm (2ft) of exposed wall studs. Non-published studies in Florida and Texas have shown treating the sill plate and the bottom 61.0cm (2ft) of the wall studs is not sufficient to protect from termite infestation (Kard pers. comm.). The penetration of the chemical at LD_{50} concentrations only reaches approximately 0.85 cm (1/3 in) into dry wood, which is not enough to provide the necessary structural protection. This type of treatment, although a labeled treatment, is not allowed as a stand-alone pre-or-during-construction treatment in some states.

Other preservative treatments used for the prevention of termite infestation include treatment with creosote and pentachlorophenol to utility poles, railroad ties, and wharf pilings, to name a few. If applied at high enough concentrations and treatment depth is complete, these treatments afford protection from *R. flavipes* and *C. formosanus*. However, *C. formosanus* will attack and penetrate wood treated at too low a concentration of these products. Chromated Copper Arsenate (CCA) has been used for decades but was banned by the EPA in 2004. Alkaline Copper Quaternary (ACQ) is a newer substance used to treat wood to prevent fungus rot and termite infestation. An economic drawback to using ACQ is that copper is relatively expensive, thereby increasing the price of the final product (Morrison 2004). ACQ has been shown to have 100% mortality against *C. formosanus* when the termites were fed treated wood (Lee et al. 2005). A study on thermal modification of ACQ treated wood showed that for some wood species palatability of treated wood was equal to non-treated wood, and in one wood species termites preferred the treated wood over non-treated wood (Shi et al. 2007). Care must be taken when disposing of wood treated with creosote, pentachlorophenol,

CCA, or ACQ. The wood should be disposed of in a landfill but should not be burned, as toxic chemicals may be released.

Construction Techniques to Build-out Termites

Termites need moisture, food, and shelter to survive. Utilizing modified construction techniques and termite-resistant building materials will make wooden structures less vulnerable to attack by termites.

Moisture: Because subterranean termites require moisture to survive, it is imperative the amount of moisture under and surrounding a structure be kept to a minimum. Rain gutters and downspouts should be used, and the slope of sidewalks, patios, and driveways should be such that water is directed away from the structure (Lstiburek and Carmody 1993). The soil around landscaping should not become saturated and any plumbing should be in good repair. If a house has a crawlspace, a vapor barrier should be placed over approximately 75% of the soil surface. This may need to be adjusted according to how the lumber in the structure reacts. Too little moisture will dry and split the lumber and too much moisture may cause swelling and rot. Proper ventilation of the crawl space is important for humidity reduction. Building codes generally require vent openings of at least $0.1m^2$ per $14m^2$ of horizontal crawlspace area (Potter 2004).

Food source: Trash from construction waste is often left behind on the ground under the foundation. This trash may consist of cardboard, paper, wooden marking stakes, and scrap lumber. These items serve as a food source for termites and should be removed before the foundation is poured. No wooden parts of the structure should ever be in direct contact with the soil. Cellulose-containing materials such as fencing, landscaping timbers, and firewood should not be placed against the foundation of the house. Not only

do these materials supply a food source but they may allow termites non-detected access into a structure.

One method of constructing homes that are less conducive to termite attack is the utilization of non-cellulose building materials. Replacing traditional wood framing with steel framing helps reduce termite infestation. Another option to eliminate wood framing is to use a system such as Tridipanel® (E.V.G., Austria). This system utilizes prefabricated polystyrene panels with wire mesh to build the walls of a structure. Panels are assembled and then shotcreted with a non-cellulose masonry product. According to the manufacturer this provides a termite-proof structure (Hadrian Tridi-Systems 2008). While this system does not contain materials nutritionally valuable to termites, it is non-toxic and will not stop termites from entering the structure through cracks or utility penetrations. Once within the wall of the structure termites can tunnel through the polystyrene panels to gain access to any cellulose material contained elsewhere within the structure.

A method of home construction used in Florida is the construction of concrete block load-bearing exterior and interior walls. Concrete offers no nutrition for termites and makes penetration of the structure by termites difficult.

Protection from the Environment:

The Council of American Building Officials began mandating the use of rigid-foam insulation on building foundations in 1992. An increase in energy savings was the purpose for this decision. As the use of this method of insulating spread across the USA, the amount of termite damage to these type of structures increased, particularly in the southern states (Smith and Zungoli 1995a, b, Williams and Bergstrom 2005). Below-

ground rigid-foam insulation affords termites protection from environmental and non-environmental factors. Foam insulation also increases temperature and may increase humidity next to the structure (Gooch 2000). The foam also provides protection from pesticide applications and makes it difficult to see termite activity when a structure is inspected (Ogg 1997). Some pesticide applicators refuse to treat or guarantee treatment of homes with below-ground foam insulation (Smith and Zungoli 1995a). One method of combating this problem is to leave a gap without insulation along the exterior walls from ground level and extending upward 15cm (6in), to facilitate visual inspection. Treating the foam with DOT is another option in those areas where building codes demand the use of insulation below ground. Williams and Bergstrom (2005) found that only 3.2% of expanded polystyrene rigid foam insulation treated with DOT showed termite damage after three years.

Biological Control

The greater emphasis today of using non-chemical control methods has caused an increase in the study of use of biological control measures. The concept of using biological control is not new and knowledge and studies on the effects of various pathogens against termites have existed for over 40 years (Snyder 1935, Yendol and Paschke 1965). Common biological control measures employ natural enemies of termites such as parasites, pathogens, or predators (Grace 1997). Recent studies have shown the detrimental effects of various entomopathogenic fungi and bacteria on termites (Neves and Alves 2000, Osbrink et al. 2001b, Dong et al. 2007, Maketon et al. 2007). Dampwood termites infected with entomophillic nematodes have been shown to modify their behavior, and behavioral change may protect the colony from further exposure to

the microorganism (Wilson-Rich et al. 2007). This modification of behavior is a major reason why biological control has not been successful. Termites will segregate infected nestmates and may remove them from the colony. Termites have been subjected to these natural attacks for centuries, and it may take some modification of these biological control measures to make them effective on a large scale.

Not only do termites impact humans by attack on structures but they also attack agricultural crops. The movement toward organically grown crops has necessitated study into the use of biological control measures in agricultural settings. Development of termite resistant cultivars combined with appropriate cultural techniques may be the best way to obtain the goal of minimizing termite impact with minimal chemical use (Logan et al. 1990).

Baiting Systems

An effective method of termite control is the use of baiting systems (Forschler and Ryder 1996, Haagsma and Bean 1998, Getty et al. 2000, Prabhakaran 2001, Su et al. 2004b, Riegel et al. 2005, Getty et al. 2007). There are variations of these systems but all have a cellulose bait matrix containing a slow-acting, non-deterrent poison. To be most effective, the poison must not modify termite behavior at sub-lethal doses and it must have a dose-dependent lethal time (Su and Scheffrahn 1996). Foraging termites gather poison-impregnated food at a bait station and return the poison to the colony where it is distributed via tropholaxis, coprophagy, mutual grooming, and cannibalism (Suarez and Thorne 2000, Lewis and Power 2006). Factors affecting the effectiveness of bait are the quantity and form of bait being offered along with the frequency of inspection disturbance (Evans and Gleeson 2006).

Seasonal changes result in fluctuations in foraging activity of termites as well (Haagsma and Rust 1995, Houseman 1999). When termites are more active, colony elimination may occur faster than when the colony is less active. Temperature and moisture can also affect the bait, as the effectiveness of the toxins may decrease at certain temperatures. Also, excessive moisture may cause deterioration of the bait or allow fungal growth that may repel termites (Spomer and Kamble 2005, Heintschel et al. 2007).

There are two types of bait systems utilized today: above-ground and in-ground. Above-ground stations are used inside buildings where a termite colony has established itself in the upper floors of the structure, and the colony has limited or no contact with soil. Above-ground stations are placed directly over mud shelter tubes to expose termites to the bait. In-ground stations are buried in the ground at fixed intervals along the periphery of a structure. Only station tops are visible. In-ground stations are usually cylindrical and have a removable top to allow access to the internal cellulose bait matrix by the person servicing the station. Stations also have openings incorporated into their sidewall to allow access by foraging termites. Subterfuge® termite bait (BASF, Research Triangle Park, NC) uses a cellulose matrix containing hydramethylnon. This matrix is placed in the station at the time of installation, thus limiting disturbance of the station as no chemical needs to be added later upon discovery of an infestation. The Exterra® Termite Interception and Baiting System (Ensystex, Fayetteville, NC) incorporates wooden slats called 'interceptors' into its bait station perimeter. When termite activity is noted upon inspection, diflubenzuron bait matrix is inserted into the device. This is meant to minimize disturbance and reduce chemical use as it is only placed in active devices. The latest edition to the Exterra system places active bait into the station at the

time of installation. The Advance™ Termite Bait System (Whitmire Micro-Gen, St. Louis, MO) has a wooden food source at the bottom of the station and bait cartridges in top. This system also is meant to minimize disturbance as bait cartridges can be replaced as needed without disturbing the wooden food in the station bottom. In the USA, the Sentricon® Colony Elimination System (Dow AgroScience, Indianapolis, IN) is the most widely used baiting system. Initially stations contain bait composed of two wooden slats containing no toxin/active ingredient. Stations are monitored for termite activity and when activity is discovered the wooden slats are replaced by a cellulose bait matrix containing noviflumuron. To minimize disturbance, additional stations containing noviflumuron may be installed around the infested station, without removing the cellulose material and replacing it with a bait cartridge. To minimize disturbance and increase the speed of station inspections, Dow Agrosciences has developed the Electronic Sensing Protection (ESP™) detection unit. The initial wooden slats are affixed with an electricity-conducting strip and top end sensor that is scanned by the ESP unit. When the unit is swept over the station, the ESP unit emits different beeps indicating whether or not the station is active.

The modes of action of chemicals used in termite baits fall into two categories, insect growth regulators (IGR), and slow-acting metabolic inhibitors and neurotoxicants (Cabrera et al. 2002). An IGR affects the insect by targeting the development and growth of the termite. Diflubenzuron, hexaflumuron, and noviflumuron are all chitin synthesis inhibitors used in baiting systems and are non-repellant and effective at low concentrations (Karr et al. 2004, King et al. 2005, Su 2005, Cabrera and Thoms 2006, Husseneder et al. 2007, Vahabzadeh et al. 2007). Neurotoxicants used in baiting systems

are hydramethylnon or sulfluramid, which act as stomach poisons. Su and Scheffrahn (1996) showed that colonies exposed to bait with sulfluramid were only partially suppressed after 12 months, while colonies exposed to hexaflumuron were eliminated.

One advantage of baiting systems is that only a few grams of active ingredient are needed to control an infestation. Concrete foundations are not drilled and landscape near the foundation is not disturbed (Su 1994). The main disadvantage to this type of system is cost. The requirement for regular monitoring and servicing of the stations results in an increased cost to the homeowner. Loading every station with hermetically-sealed bait would reduce the frequency of needed monitoring and thus could reduce the costs (Su 2007).

Termite Population and Foraging Studies

The success of termite baiting systems relies on knowledge of termite biology and behavior, but this knowledge is difficult to gain due to the subterranean colonies. For baits to work effectively it is important to know the colony's number of foraging termites and foraging area.

Foraging Populations: The two most common methods of estimating foraging populations are the Lincoln index and the weighted means model. Su (1993) estimated the number of foraging *R. flavipes* in a Florida colony to be as many as 5 million, and Grace et al. (1989) estimated the number of foragers in a Toronto colony to be ca. 3.2 million. In Georgia, the foraging populations of individual colonies of *R. virginicus* and *R. hageni* numbered ca. 154,000 and 48,000 respectively (Forschler and Townsend 1996). A study of *Heterotermes aureus* foragers in Arizona estimated their number to be over 300,000 (Baker and Haverty 2007). *Reticulitermes* sp. foraging populations in

California were estimated to have as many as 194,000 members (Haverty et al. 2000). Colonies of *C. formosanus* may contain 6,800,000 individuals (Su and Scheffrahn 1988).

Foraging Territory: The size of the foraging territory is also of interest when gathering information for effective baiting. Bait stations do not form a continuous chemical barrier in the soil but instead rely on foragers transporting the poison to the colony. If the bait is spaced improperly around a structure or a non-adequate number of stations used, the colony may not be completely eliminated. Seasonal differences are important factors that affect foraging behavior of termites, and different species react differently to these factors (Jones 1988, Haagsma and Rust 1995, Evans 2001, Glenn 2005). The size of the foraging territory for a termite colony may range from 9.0 to 2,361m^2 (Jones 1990, Su et al. 1993, Brown et al. 2008).

Previous Work on the Tallgrass Prairie Preserve

Brown et al. (2008) characterized three colonies of *R. flavipes* on an open tallgrass area within the TGPP. Estimated foraging ranges for the three colonies were 9.0, 24.8 and 92.3m^2, with estimated foraging populations of 36,302, 183,493 and 76,812 individuals, respectively. Soldiers comprised 4.46, 3.65 and 2.69% of these foraging populations, respectively.

Taxonomy

Morphological Identification: Morphological dichotomous keys for identification of alates and soldiers of *Reticulitermes* sp. have existed since 1920 and have been updated through the years (Banks 1946, Gleason and Koehler 1980, Scheffrahn and Su 1994, Brown et al. 2005). However, due to overlapping measurements for the same

morphological characteristics between *R. flavipes* and *R. virginicus,* identification and separation of these two species remains difficult (Brown et al. 2005).

Molecular Techniques for Termite Identification: Utilization of molecular techniques for identification of termites has been used for approximately 10 years. Foster et al. (2004) used an AT-rich region of mitochondrial DNA (mtDNA) to identify *R. flavipes*. The mtDNA cytochrome oxidase II (COII) gene was used to support taxonomic designations of *Reticulitermes* sp. in California (Copren et al. 2005). Su et al. (2006) identified Chilean *Reticulitermes* species by sequencing portions of the COII, and mtDNA 12S and 16S rRNA genes. The 16S gene is the most commonly used in termite molecular taxonomy, and was used to identify *Reticulitermes* sp. in Delaware, Maryland, Oklahoma, and Oregon (McKern et al. 2006, King et al. 2007), and also to describe and validate a new species in Delaware, Georgia, Maryland, North Carolina, South Carolina, the western USA and Canada (Szalanski et al. 2006, Austin et al. 2007). Additionally, genetic variation within *Reticulitermes* species collected in North America, Eastern Europe and the Middle East was examined (Austin et al. 2004, Austin et al. 2005, Austin et al. 2006, Tripodi et al. 2006, McKern et al. 2007).

CHAPTER III

MATERIALS AND METHODS

Taxonomy

Morphological Identification: Termites collected from the study areas were preserved in 100% ethyl alcohol. Standard taxonomic dichotomous keys were used to identify the termites to species (Banks 1946, Gleason and Koehler 1980, Scheffrahn and Su 1994, Brown et al. 2005).

Molecular Techniques

Termites were also identified using Polymerase Chain Reaction (PCR) techniques. Two termites from each sample of alcohol preserved specimens were placed on filter paper and allowed to dry at room temperature. The DNA of whole workers from each sample group was extracted using a Qiagen DNeasy® Tissue Kit (Qiagen Sciences, Germantown, MD). The extract was quantified utilizing the ND-1000 nanodrop spectrophotometer located in the OSU Biochemistry Microarray Core Facility. Extracts with a 260/280 ratio below 1.6 or with a mass <10ng/µl were discarded. The extracts were amplified utilizing FastStart PCR Master® (Roche, Indianapolis, IN). PCR primers known to amplify a ≈ 428bp region of the mtDNA 16S rRNA gene in *Reticulitermes* sp. were used. These primers are LR-J-13017 (5′-TTACGCTGTTATCCCTAA-3′) (Austin et al. 2004) and LR-N-13398 (5′-CGCCTGTTTATCAAAAACAT-3′) (Austin et al.

2004). The product was cleaned of excess dNTPs and primers using ExoSAP-IT® (USB, Cleveland, Ohio), and a combination of the hydrolytic enzymes Exonuclease I and Shrimp Alkaline Phosphatase. A sample of the resulting product was submitted to the OSU Biochemistry Microarray Core Facility for sequencing. Sequencing was performed with the Applied Biosystems BigDye® terminator cycle sequencing kit version 1.1 using standard protocols and analyzed with an Applied Biosystems Model 3730 DNA Analyzer. The resulting sequence was submitted to the National Center for Biotechnology Information (NCBI) website and compared with known sequences utilizing the Basic Local Alignment Search Tool nucleotide collection (BLASTn). Upon verification of correct morphological identification via BLASTn, a consensus sequence was identified using the ClustalW program at EMBL-EBI (European Bioinformatics Institute). The consensus sequences used for all subsequent molecular identifications were: AY257235.2 (*R. hageni*), AY441992.1 (*R. tibialis*), DQ001971.1 (*R. flavipes*). A consensus sequence was also identified for *R. virginicus*: EU259775.1.

Study Location

The Nature Conservancy's Oklahoma Tallgrass Prairie Preserve (TGPP), located 89km northwest of Tulsa, OK, in Osage County served as the study location. The preserve encompasses 15,659 hectares of land consisting mainly of native tallgrass prairie, with a north-to-south central swath of Cross Timbers that begins in North Central Kansas and extends to Central Texas. The Cross Timbers swath contains both prescribed-burn sites and no-burn sites managed by the Nature Conservancy and range scientists from Oklahoma State University. Bison roam freely on the TGPP but are excluded from a 142 hectare area that includes the Cross Timbers.

Monitoring Devices

In-ground stations consist of cylindrical 10.2cm inside diameter (i.d.) polyvinyl chloride (PVC) pipe cut to 20.3cm (8in) lengths. Each pipe has four-equally spaced parallel longitudinal rows of twelve 3.2mm (0.125in) diameter holes. Drill holes begin 1.3cm (0.5in) from one end of the pipe and are spaced 1.3cm (0.5in) apart. The pipe is vertically inserted into a 17.8cm (7.0in) deep hole in the soil pre-drilled with a gas-powered auger equipped with a 10.2cm (4.0in) diameter bit. A wood 'sandwich' consisting of seven parallel, rectangular 17.8 x 6.4 x 0.6cm (7.0 x 2.5 x 0.25in) pine sapwood slats, each separated by a flat wooden tongue depressor and bound with nylon 'zip' ties, then wrapped with a rectangular 37.5 x 18.5cm (14.8 x 7.3in) section of corrugated cardboard, was inserted into each pipe (Figs. 3.1a,b). A standard 10.2cm (4.0 in.) diameter PVC cap was placed on top of each pipe to exclude sunlight, moisture and animals, but is removable to facilitate inspection of the device (Fig. 3.1c) (Brown et al. 2004).

Soil-surface rectangular ground-boards of fir/spruce/pine, each measuring 30.5 x 15.2 x 2.5cm (12.0 x 6.0 x 1.0in) were placed flat on bare soil. A standard, solid building brick was placed on top of each board to reduce disruption or loss by wind and animal activity (Fig. 3.1d)

Study Sites

Three study sites were established. Site 1 was established on the prescribed-burn area, and Sites 2 and 3 on the no-burn area. The prescribed-burn area supports plant life comprised mainly of grasses and thinly scattered trees. The predominant plants and grasses found on this area are the legume live goat's rue [*Tephrosia virginiana* (L.),

Pers.], and the grasses Indiangrass [*Sorghastrum nutans* (L.) Nash], switchgrass (*Panicum virgatum* L.) and big bluestem (*Andropogon gerardii* Vitman). Because of cyclic prescribed-burns, few mature trees are found on this area. Blackjack oak [*Quercus stellata* (Wangenh.)] and [*Q. marilandica* (Münchh.)] regenerates on this area, but most are ca. 1.5m in height, clustered and shrub-like in appearance.

On the prescribed-burn area, 25 in-ground stations were initially installed as a 12.0 x 12.0m square grid. These stations were configured in straight lines with a "checkerboard" arrangement spacing of 3.0m between stations. Additionally, a 9.0 x 9.0m square grid of 20 soil-surface ground-boards was overlaid in such a way that each surface ground-board is centered between four in-ground stations**.** The result was a total of 45 monitoring devices, each subtending an area of 4.5m^2. When stations ≤6m from the border of the grid became active with termites, additional stations and ground-boards were added to expand the grid border to encompass the active termite colony. The prescribed-burn site was eventually expanded to 136 in-ground stations and 122 soil-surface ground-boards (258 monitoring stations) on a 39.0 x 30m grid (Fig. 3.2). Three colonies were identified within this area: summer burn sites 2005 (SBS05), 2006 (SBS06), and 2007 (SBS07).

Two additional grids were established on Sites 2 and 3 within the cross-timbers no-burn area. This area is populated by a mature stand of blackjack oak averaging 6-8m in height. The grid on Site 2 consists of 25 in-ground stations and 25 soil-surface ground-boards (Fig. 3.3). The spacing is 3.0m between stations, the same as the prescribed-burn site. The Site 3 grid consists of 50 in-ground stations installed on a 27.0 x 12.0m rectangular grid, also with 3.0m spacing between stations. Additionally, a 27.0 x 12.0m

rectangular grid of 50 soil-surface ground-boards was overlaid resulting in a total of 100 monitoring devices. The overlay spacing is the same as the prescribed-burn site, and was expanded as needed to encompass new termite activity. This grid eventually expanded to 75 in-ground stations and 82 soil-surface ground-boards (Fig. 3.4). Two colonies were identified within this area: Cross Timbers Sites 2006 (CT06) and 2007 (CT07).

Delineation of Foraging Areas

A triple-mark-release-recapture (TMRR) technique (Haverty et al. 2000) was used to delineate termite foraging territories. The cardboard wrapped 'sandwich' of a station with active termites was removed and placed in a plastic container, then a new cardboard-wrapped 'sandwich' was placed into the station. The plastic container containing the collected termites, cardboard and 'sandwich' was taken to the laboratory and placed on a plastic tray. A low-pressure aspirator was used to aspirate the termites into a collection tube. Termites were then counted and sorted according to caste (worker, soldier or alate), and ca. 500 collected termites were placed in a 10.2cm diameter x 6.4cm (4in diameter x 2.5in) tall plastic container. Two pieces of Whatman® #1 90mm diameter (3.5in) filter paper previously impregnated with 0.1% (wt/wt) Nile Blue A dye (Aldrich, Milwaukee, WI) were moistened with reverse-osmosis water, pressed between two paper towels to remove excess water and placed in each container. Termites were placed into the container and placed under dark conditions at 22℃ with 95+% humidity, and allowed to feed on the filter paper for 14 days. Termites were then recounted to determine the number of dyed and non-dyed termites to be returned to the field. On the 15th day the plastic containers containing the termites were transported to the field, the filter paper removed, and the group of dyed and non-dyed termites placed into the station from which

they were originally collected. After two weeks, the contents of any monitoring stations surrounding the original station containing blue termites were collected, all termites counted and the number of dyed and non-dyed termites determined. The TMRR process was repeated twice. During the second and third TMRR collections, termites were counted according to caste and color: non-dyed worker, soldier, and alate, and dyed-worker, soldier and alate. Subsequent TMRR colonies were fed filter paper impregnated with 0.5% (wt/wt) Neutral Red dye (Aldrich, Milwaukee, WI) (Su et al. 1983). Using different dyes ensures different colonies can be separated.

Foraging Population Estimates

Both Lincoln index and weighted means model calculations were used to estimate the number of foraging termites for each colony studied (Begon 1979, Grace et al. 1989, Su 1993, Haverty et al. 2000, Brown et al. 2008). The Lincoln index, *M*=number of marked termites released, *n*=total number of termites recaptured, and *m*=marked termites recaptured, was used to estimate a colonies' number of foraging termites:

$$\hat{x} = M_1 n_1 / m_1$$

$$SE = \sqrt{M_1^{\,2} n_1 (n_1 - m_1) / m_1^{\,3}} \,.$$

For the weighted means model, *M*=number of marked termites released, *n*=total number of termites recaptured and *m*=marked termites recaptured:

$$\hat{p} = (\sum m_i n_i) / [(\sum m_i) + 1] \,.$$

$$SE = \sqrt{[1/(\sum m_i + 1)] + [2/(\sum m_i + 1)^2] + [6/\sum m_i + 1)^3]} \,.$$

Foraging Population Comparisons

A comparison set of termite foraging populations on the burned areas to the non-burned areas was made. Assume there are three burn values, whose foraging population values are denoted by L_{11}, L_{12}, and L_{13}, and two non-burn values, whose foraging population values are denoted by L_{21} and L_{22}. The null hypotheses tested was:

H_0: average of burn values = average of non-burn values.

This translates to the following hypothesis:

$$H_0 : 2 \times L_{11} + 2 \times L_{12} + 2 \times L_{13} = 3 \times L_{21} + 3 \times L_{22}$$

If this situation is considered to be analogous to performing a contrast, the coefficients would then be 2, 2, 2,-3 and -3 for the values L_{11}, L_{12}, L_{13}, L_{21} and L_{22}, respectively. Let's allow estimates for L_{ij} to be1_{ij}, and the standard error of 1_{ij} to be s_{ij}. By Steel and Torrie (1980) the test for a contrast would be $t=Q/S_Q$ where,

$$Q = 2 \times l_{11} + 2 \times l_{12} + 2 \times l_{13} - 3 \times l_{21} - 3 \times l_{22} \text{ and } S_Q = s\sqrt{\sum c_i^2 / r}\,.$$

The value of s is a pooled standard deviation, the c_i's are the contrast coefficients, and r is the number of observations per treatment. A normal approximation was used to test this hypothesis and the final test statistic was:

$$Z = (2 \times l_{11} + 2 \times l_{12} + 2 \times l_{13} - 3 \times l_{21} - 3 \times l_{22}) / [(\sqrt{30})(\sqrt{(s_{11} + s_{12} + s_{13} + s_{21} + s_{22}) / 5})]\,.$$

Soldier Percentage Determinations

Soldier percentages were calculated as [soldiers/(soldiers + workers)] x 100.

Figure. 3.1. (a) In-ground monitoring station components including wood 'sandwich', cardboard, and PVC pipe, (b) assembled in-ground monitoring station (top view), (c) emplaced in-ground monitoring station, (d) emplaced soil-surface ground-board.

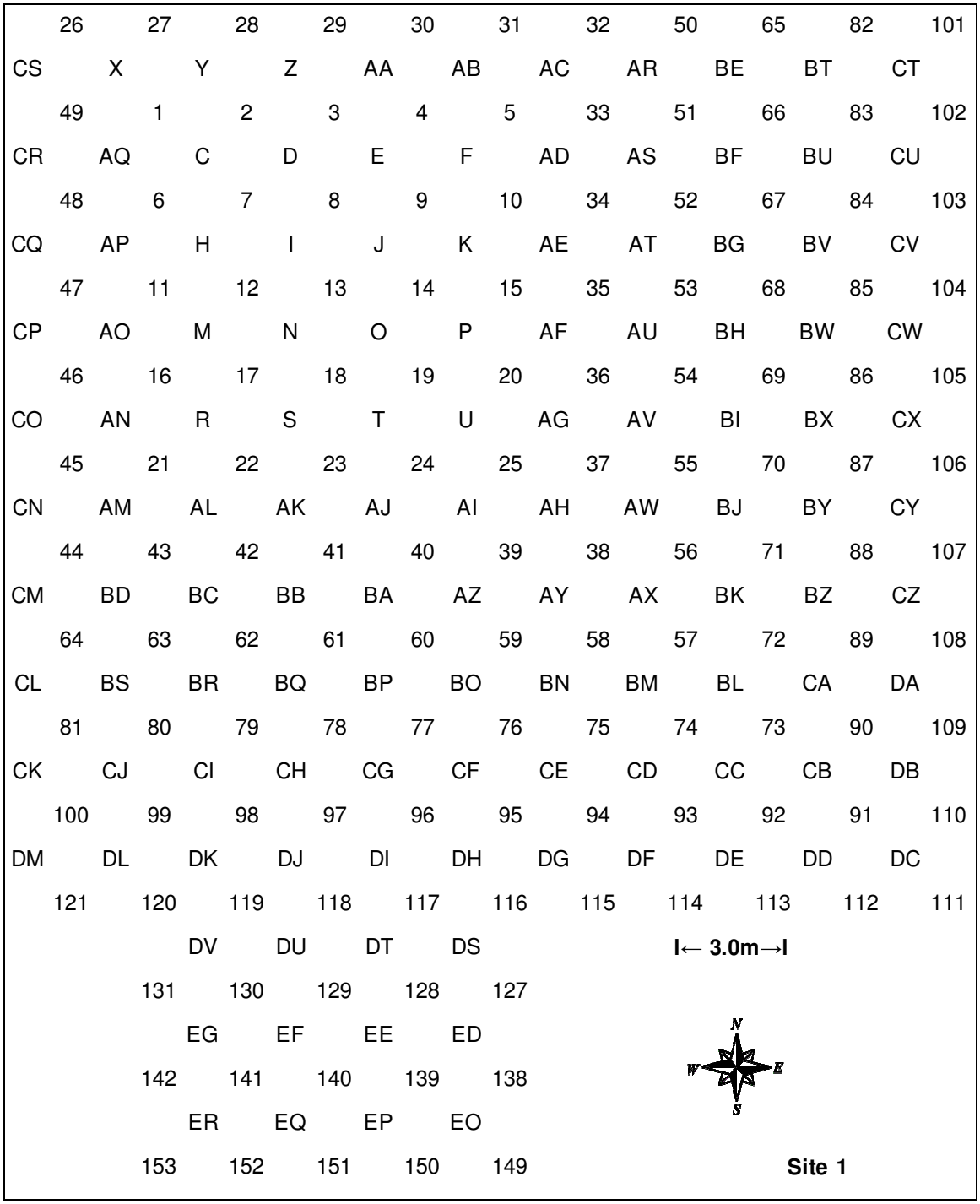

Figure 3.2. Grid layout of 258 monitoring devices on the Nature Conservancy's Tallgrass Prairie Preserve Cross Timbers prescribed-burn area. Each number denotes a 10.2cm diameter by 20.3cm deep in-ground monitoring station; letters denote a rectangular 30.5 by 15.2 by 2.5cm soil-surface ground-board. Similar devices are spaced 3.0m apart and each subtends an area of 4.5m^2.

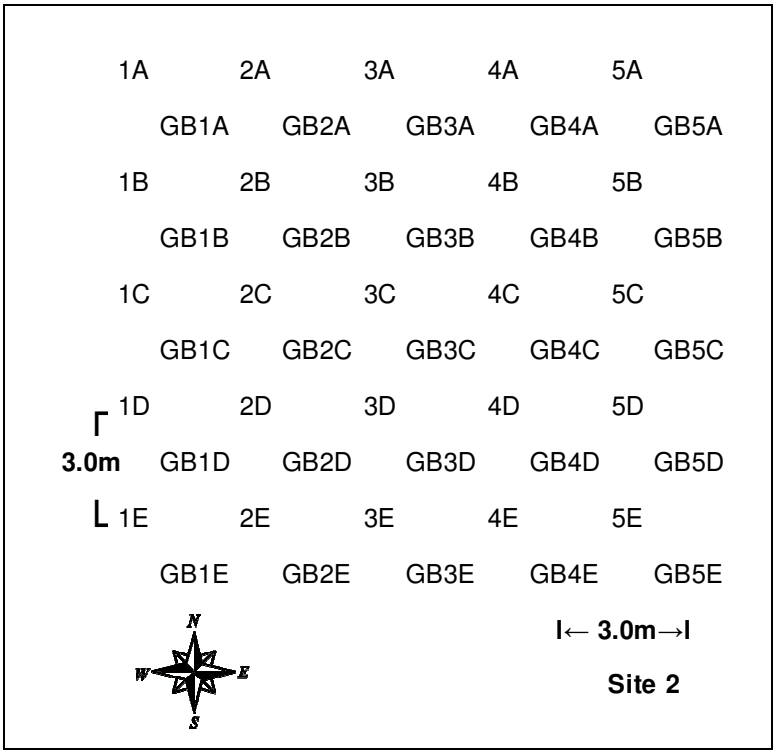

Figure 3.3. Grid layout of 50 monitoring devices on the Nature Conservancy's Tallgrass Prairie Preserve Cross Timbers no-burn area. Each number followed by a letter denotes a 10.2cm diameter by 20.3cm deep in-ground monitoring station; numbers preceded by GB denote a rectangular 30.5 by 15.2 by 2.5cm soil-surface ground-board. Similar devices are spaced 3.0m apart and each subtends an area of 4.5m^2.

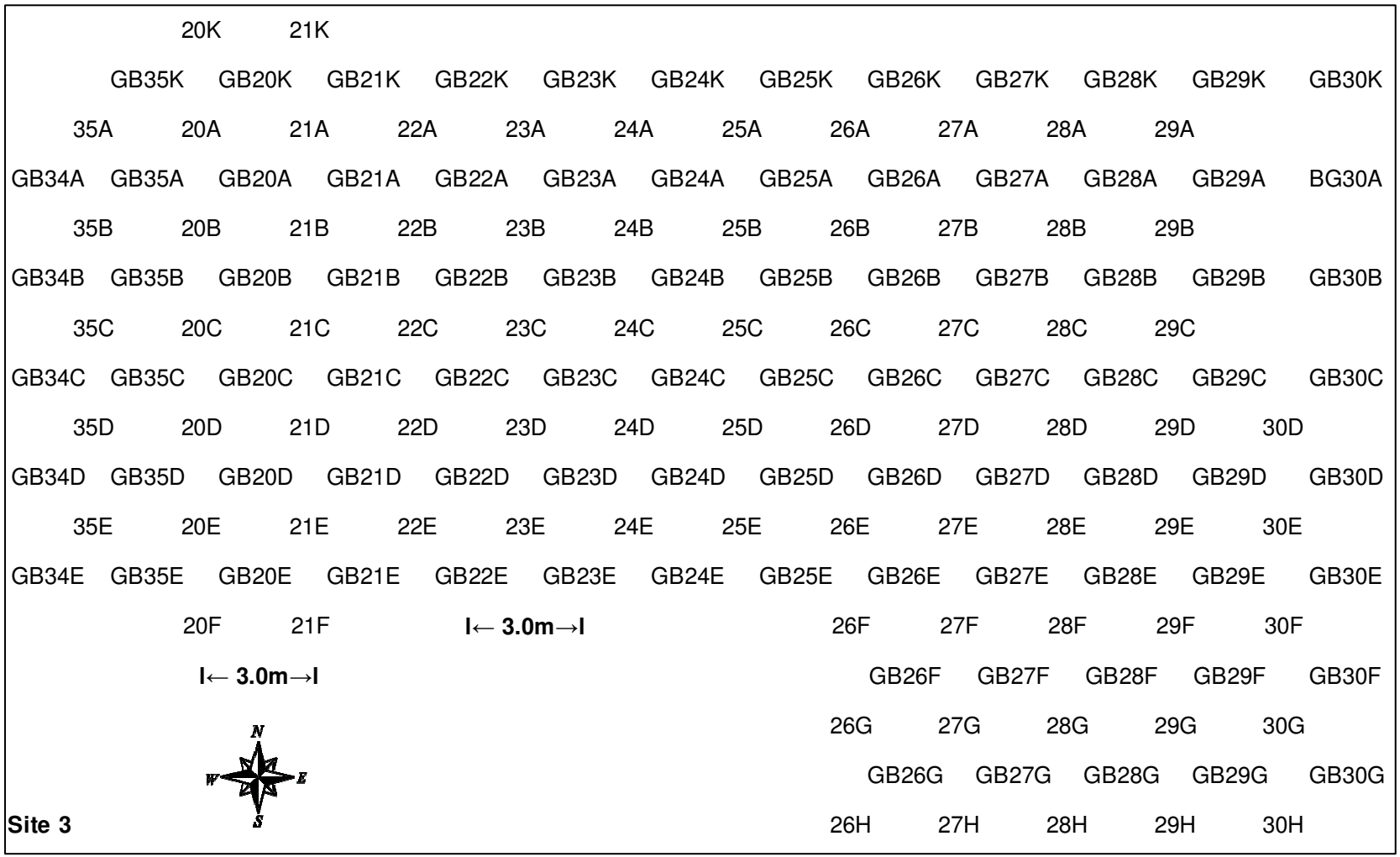

Figure 3.4. Grid layout of 163 monitoring devices on the Nature Conservancy's Tallgrass Prairie Preserve Cross Timbers no-burn area. Each number followed by a letter denotes a 10.2cm diameter by 20.3cm deep in-ground monitoring station; numbers preceded by GB denote a rectangular 30.5cm by 15.2cm by 2.5cm soil-surface ground-board. Similar devices are spaced 3.0m apart and each subtends an area of 4.5m^2.

CHAPTER IV

RESULTS AND DISCUSSION

Species Identification

Morphological identifications of *R. hageni* and *R. tibialis* soldiers were consistent with molecular identifications. Due to overlap of measurements of some body parts of *R. flavipes* and *R. virginicus* soldiers, soldiers were first tentatively identified morphologically and then positively identified molecularly as *R. flavipes*. Termites within the prescribed-burn and no-burn areas were identified as *R. flavipes* and *R. hageni*, respectively. Discovery and identification of *R. hageni* was exciting because all termites previously identified on the TGPP were *R. flavipes.* Subsequent sampling and identification after the completion of the study found *R. hageni* within the prescribed-burn area and *R. flavipes* within the no-burn area, thus both species inhabit these different vegetative habitats. Additional termites collected 10m from the prescribed-burn 2005 area were identified as *R. tibialis.* To date *R. virginicus* has not been collected on the TGPP, but it is possible this species is present. One reason that *R. virginicus* has not been collected could be that for this study termites that were collected came from 27 in-ground stations and six surface ground-boards, and it has been reported that *R. virginicus* is rarely collected from in-ground stations (Haverty et al. 1999). A recent survey of

Oklahoma termites supports this finding as no *R. virginicus* were collected from 61 in-ground stations located across southern counties (Smith 2008). The presence of two *R. hageni* colonies within the no-burn area was interesting as well. This area has a dense shade canopy of scrub blackjack oak. Also, a *R. hageni* sample collected from the prescribed-burn area came from an in-ground monitoring station located in the shade of a tree. All *R. hageni* collected in the survey by Smith (2008) were found in shaded areas, indicating *R. hageni* in Oklahoma may prefer shaded areas.

Delineation of Foraging Areas

Two foraging populations were identified within Site 3 on the no-burn area, CT06 and CT07, and three populations, SBS05, SBS06, and SBS07, were identified within Site 1 on the prescribed-burn area. Foraging territories are shown in Figures 4.1 and 4.2. Foraging territory estimates and maximum linear foraging distances are given in Table 4.1. Site 2 within the no-burn area had only 13 termites in one ground-board and none within any of the in-ground monitoring stations. It was noted that the soil on Site 2 was moister than Site 3. Dampness and mold often occurred within the in-ground monitoring devices. It is not known if there were inherently fewer termites within Site 2 or if the damp, moldy conditions within in-ground stations created unfavorable conditions that repelled termites.

Estimated foraging areas for individual colonies on the tallgrass area ranged from 9.0 to 92.3m^2 (Brown et al. 2008). The mean foraging area was 42.0m^2 compared with 27.9m^2 for the Cross Timbers area.

Foraging Population Estimates

Foraging population estimates are given in Table 4.2. Lincoln index calculations

estimated the foraging termite populations to range between 59,249 (±17,732) and 138,641 (±23,378) within the burn area, and between 27,715 (±5,831) and 127,743 (±7,373) within the no-burn area. The weighted means model estimated populations ranging between 103,093 (±7,081) and 422,780 (±19,297) termites within the burn area, and 44,179 (±4,879) to 207,141 (±9,190) within the no-burn area. The estimates derived from using these two methods never agreed. Lincoln index estimates were less than weighted means model estimates for four of the five colonies. This is consistent with findings of other studies (Haverty et al. 2000, Brown 2005). This disparity between estimates is problematic, but these two methods are the most common means of estimating a foraging population without destroying colony infrastructure through excavation.

Foraging termite estimates by Brown et al. (2008) for the tallgrass area ranged between 10,357 (±1,167) and 79,059 (±55,411) using the Lincoln index, whereas estimates using the weighted means model ranged between 36,302 (±2,523) and 183,495 (±27,995) individuals. These estimates indicate that the number of termites in a foraging population within the tallgrass area are less, compared with those on the Cross Timbers area.

It is interesting to note that although CT06 had an estimated foraging population ca. 4.6 times greater than CT07, CT07 encompassed a larger foraging area. This difference proved true for both the Lincoln index and the weighted means model. An explanation for this may be attributed to the location of the colony. CT06 was located in an area covered by the canopy of the trees. CT07 had an open area in the canopy that allowed relatively more sunlight to reach the ground. Monitoring devices 20D, GB20D and 21D

were located within this area of increased sunlight. Big bluestem was growing in this area and there were fewer tree roots. If *R. hageni* utilize the tree roots for their primary nutrition source it might explain the smaller foraging population in this area due to relatively fewer roots compared with the full canopy over CT06. As was mentioned previously, *R. hageni* in Oklahoma may prefer shaded areas. Also, *R. hageni* may prefer the cooler temperatures and perhaps moister soil found in shade, and may prefer to feed on the roots of the trees providing the shade compared with other food resources in the area. These observations contrast with those of Houseman (1999) in Texas, who showed *R. hageni* prefer warm, dry conditions. To answer these questions will require additional studies.

Contrast Analysis

Contrast analysis of the termite populations within the burned areas compared with the non-burned areas was performed. The null hypothesis tested was that the Lincoln index foraging populations determined for the prescribed-burn sites, are equal to the populations within the non-burned sites. The p-value was 0.054, indicating there is a moderate difference between these two sets of sites. We also tested the null hypothesis using the weighted means model and calculated a p-value of 0.0001, indicating a significant difference in termite foraging behavior between the burned and non-burned areas.

These results must be considered with care. Both p-values indicate a difference between the two sites. However, the reason for the difference is not clear. The original intent for the contrast analysis was to compare the termite foraging populations within a no-burn area with foraging populations within a prescribed-burn area. However, these

contrasts compared foraging populations of two different species, each found in different habitats. It is not known if these differences in foraging numbers are a result of the type of habitat within which they are found, if it is due to the variation between species, or if it is due to other non-determined factors.

Soldier Percentage Determinations

The soldier percentage, [soldiers/(soldiers + workers)] x 100, are shown in Table 4.1. Percentages for *R. flavipes* of 0.53, 1.86 and 2.08% are lower than those of 2.69, 3.65 and 4.46% recorded for the open prairie area of the TGPP (Brown 2005). Soldier percentages for mature colonies reported by Banks and Snyder (1920) and Haverty (1977) ranged from 8.4 to14%. These numbers indicate soldier percentages may vary depending upon habitat and species.

The two colonies of *R. hageni* located within the no-burn area both had soldier percentage estimates below 1.00%. This relatively small number of soldiers does not mean the number of soldiers per worker within the colony is less than that of *R. flavipes*, but may indicate a difference in where they are located within the colony's structure. No studies have been published showing soldier percentage data for *R. hageni,* thus no comparisons can be made with published data.

Soldiers comprised 2.69, 3.65 and 4.46% of the foraging populations on the tallgrass area (Brown et al. 2008), indicating soldiers may be more abundant in colonies within these open grasslands.

Conclusions

This study shows the inherent difficulties in studying an organism living in a subterranean environment. The Lincoln index and weighted means model are useful

tools because they provide an indication of the number of foraging termites in a colony and allow comparisons between colonies. Foraging population estimations for *R. flavipes* from this study are in general agreement with estimates in the literature. Soldier percentage data show that TGPP *R. flavipes* colonies contain fewer soldiers compared with ratios in other published studies. The TGPP Cross Timbers study shows areas where future research is needed if we are to better understand the biology and ecology of USA native subterranean termites and their impacts in various ecosystems. Further analyses of nutritional resources utilized by *R. hageni* within the Cross Timbers no-burn area, and a comparison of colony foraging populations and foraging areas between locations with complete tree coverage, and where the tree canopy has openings could provide us with interesting data. A study of how soil moisture and temperature affect termite foraging activity would be interesting as well. Further study of soldier percentages for both *R. flavipes* and *R. hageni*, as well as other species, would aid in better understanding of colony structure. Ultimately, this study provides information that could be useful in further comparisons of termite behavior, biology, and ecology in different habitats.

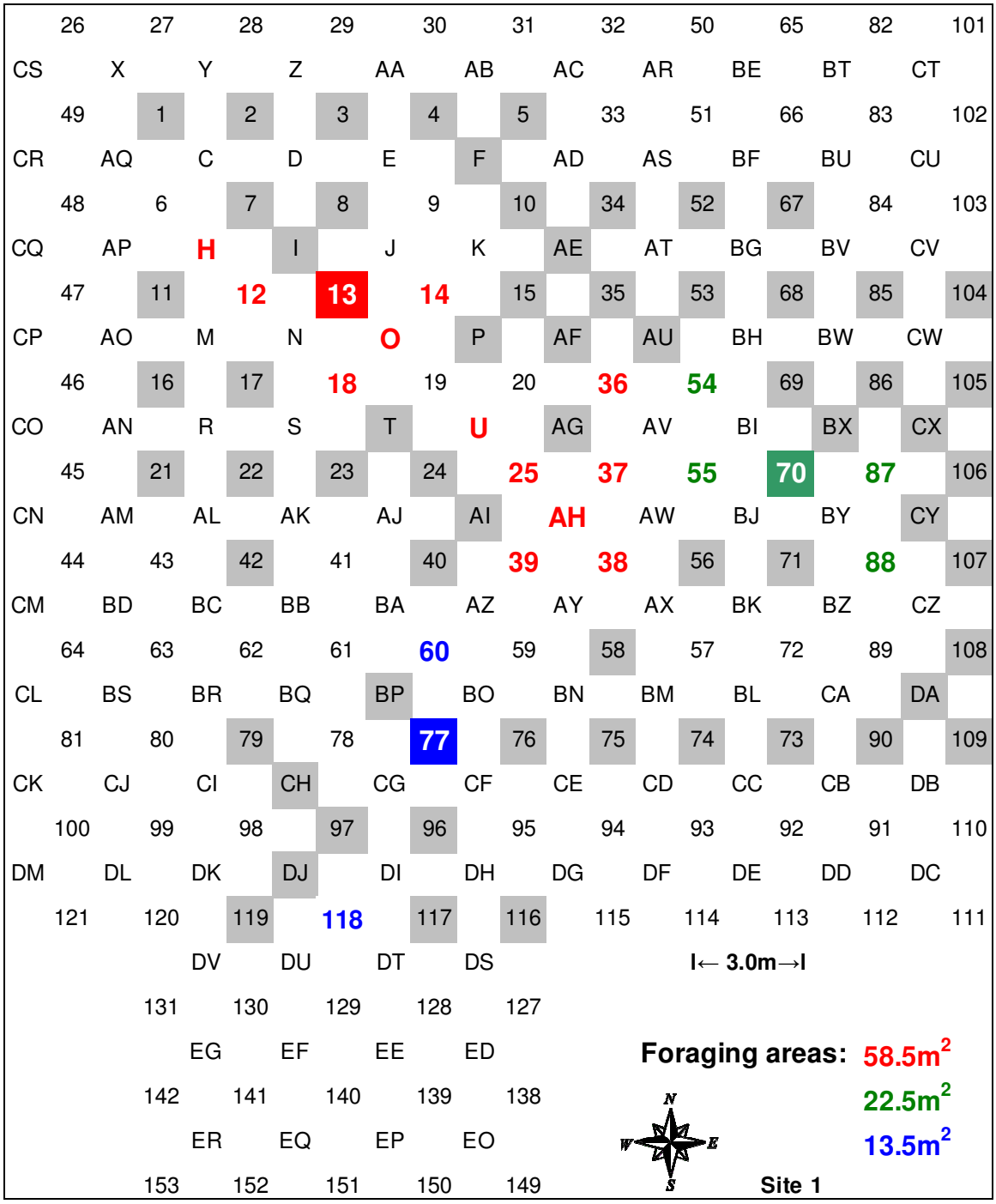

Figure 4.1. Foraging areas of three colonies of *Reticulitermes flavipes* on the Nature Conservancy's Tallgrass Prairie Preserve Cross Timbers prescribed-burn area. Each solid box with a white number represents a colony's first collection site; all same-colored numbers/letters represent subsequent collections of dyed termites. Grey shading represents stations active with non-dyed termites. Numbered sites represent in-ground monitoring stations; letters represent soil-surface ground-boards. Colors represent year of study: 2005, 2006, 2007.

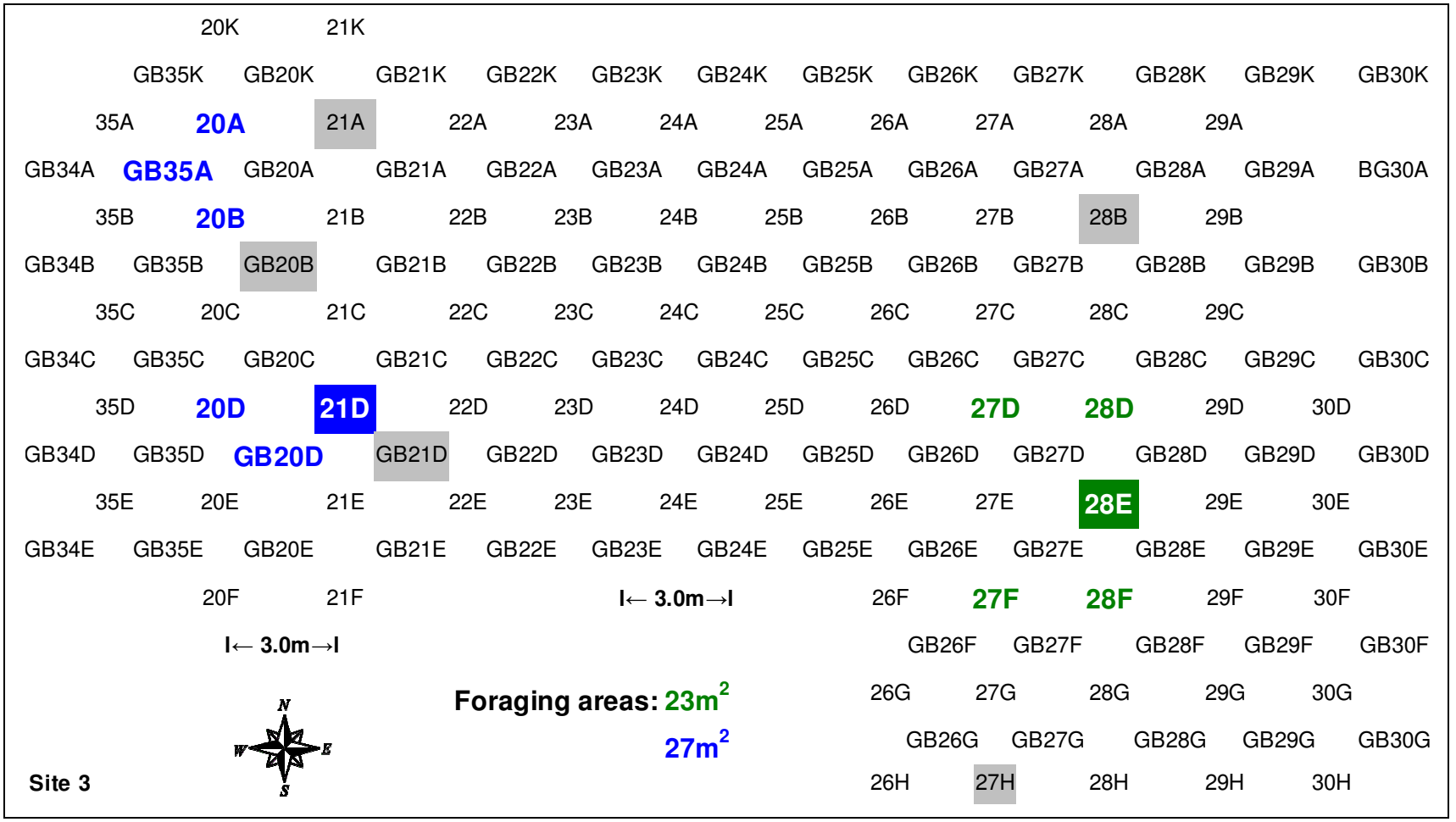

Figure 4.2. Foraging areas of two colonies of *Reticulitermes hageni* on the Nature Conservancy's Tallgrass Prairie Preserve Cross Timbers no-burn area. Each solid box with a white number represents a colony's first collection site; all same-colored numbers represent subsequent collections of dyed termites. Grey shading represents stations active with non-dyed termites. Numbered sites represent in-ground monitoring stations; numbers preceded by GB represent soil-surface ground-boards. Colors represent year of study: 2006, 2007.

Colony	Number of active monitoring devices	Foraging territory m^2	Maximum linear foraging distance
SBS05*†	13	58.5	17.0
SBS06	6	22.5	8.5
SBS07	3	13.5	9.5
CT06‡	5	18.0	6.7
CT07	6	27.0	10.5

*SBS =prescribed-burn area, and CT=no-burn area.
05, 06 and 07 are the year of study, e.g. 05=2005.
†SBS colonies are *R. flavipes*. ‡CT colonies are *R. hageni*.

Table 4.1. Foraging areas and maximum linear foraging distance of five colonies of subterranean termites (*Reticulitermes* sp.) on the Nature Conservancy's Tallgrass Prairie Preserve Cross Timbers.

Table 4.2. Average soldier percentages, and foraging population estimates (Lincoln index and weighted means model) of five colonies of *Reticulitermes* sp. on the Nature Conservancy's Tallgrass Prairie Preserve Cross Timbers.

Colony	Station ID	Mark-release-recapture cycle[a]									Soldier percentages	Lincoln index (SE)	weighted means model (SE)
		M1	n1	m1	M2	n2	m2	M3	n3	m3			
SBS05[b]	13	381	774	16	500	1170	54	949	2113	29	2.21	112342	
	14	0	2564	2	1947	0	0	0	0	0	1.72	(18692)	
	36	0	2259	4	1569	527	31	446	910	17	3.06		
	37	0	2099	4	1661	0	0	0	0	0	1.72		
	38	0	1163	2	470	117	11	77	4200	47	1.95		
	39	0	376	2	0	2273	93	499	2967	39	1.81		
	H	0	1380	6	844	0	0	0	0	0	1.49		
	12	0	0	0	0	377	48	310	0	0	5.57		
	AH	0	0	0	0	1163	29	894	0	0	1.12		
	U	0	0	0	0	427	5	317	0	0	1.81		
	18	0	0	0	0	0	0	0	1449	19	0.41		
	25	0	0	0	0	0	0	0	1414	12	1.06		
	O	0	0	0	0	0	0	0	1224	11	0.41		
	Total	381	10612	36	6991	6054	271	3492	14277	174	1.86[c]		422780 (19297)
SBS06	70	667	256	1	140	0	0	0	0	0	3.47	138641	
	54	0	3728	14	2765	2150	99	1776	337	39	1.96	(23378)	
	55	0	2153	10	1321	0	0	0	0	0	0.98		
	87	0	247	3	115	0	0	0	364	32	3.11		
	88	0	891	7	308	0	0	0	0	0	2.69		
	107	0	0	0	0	0	0	0	114	8	2.63		
	Total	667	7275	35	4649	2150	99	1776	815	79	2.08		103093 (7081)

[a]Numbers (1-3) indicate mark-release-recapture-cycle. M indicates the number of marked termites released, n indicates the number of termites recaptured (marked plus unmarked), and m indicates the number of marked termites recaptured.
[b]SBS =prescribed-burn area and CT=no-burn area. 05, 06 and 07 are the year of study, e.g., 05=2005.
[c]Mean of values immediately above.

Table 4.2. cont. Average soldier percentages, and foraging population estimates (Lincoln index and weighted means model) of five colonies of *Reticulitermes* sp. on the Nature Conservancy's Tallgrass Prairie Preserve Cross Timbers.

Colony	Station ID	Mark-release-recapture cycle[a]									Soldier percentages	Lincoln index (SE)	weighted means model (SE)
		M1	n1	m1	M2	n2	m2	M3	n3	m3			
SBS07[b]	77	876	0	0	0	0	0	0	431	4	0.36	59249	
	60	0	655	5	584	2882	15	2309	328	6	0.44	(17732)	
	118	0	89	6	26	153	2	153	0	0	2.89		
	Total	876	744	11	610	3035	17	2462	759	10	0.53[c]		212224 (34409)
CT06	28E	10285	2090	179	1868	38	10	5	0	0	0.86	127743	
	27D	0	714	41	505	13	5	9	0	0	1.93	(7373)	
	28F	0	624	56	548	0	0	0	460	19	1.11		
	27F	0	0	0	0	3915	181	3379	39	4	1.14		
	28D	0	0	0	0	0	0	0	586	14	0.51		
	Total	10285	3428	276	2921	3966	196	3393	1085	37	0.96		207141 (9190)
CT07	21D	725	45	5	42	0	0	0	0	0	0.23	27715	
	20B	0	471	13	372	0	0	0	0	0	1.91	(5831)	
	20D	0	325	4	296	0	0	0	0	0	0.31		
	20A	0	0	0	0	226	31	215	1052	21	0.55		
	GB20D	0	0	0	0	35	3	24	0	0	0		
	GB35A	0	0	0	0	434	6	326	0	0	1.61		
	Total	725	341	22	710	695	40	565	1052	21	0.76		44179 (4879)

[a]Numbers (1-3) indicate mark-release-recapture-cycle. M indicates the number of marked termites released, n indicates the number of termites recaptured (marked plus unmarked), and m indicates the number of marked termites recaptured .

[b]SBS =prescribed-burn area and CT=no-burn area. 05, 06 and 07 are the year of study, e.g., 05=2005.

[c]Mean of values immediately above.

CHAPTER V

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VITA

Matthew Paige Smith

Candidate for the Degree of

Master of Science

Thesis: SUBTERRANEAN TERMITES OF THE OKLAHOMA TALLGRASS PRAIRIE PRESERVE CROSS TIMBERS

Major Field: Entomology

Biographical:

Personal: Born in Peoria, IL. Son Zachary Paige Smith was born July 25, 1989. Married Anita Lynn Miller on October 8, 1993.

Education: Graduated from East Peoria Community High School, East Peoria, IL. Received Bachelor of Science degree in Entomology from Oklahoma State University in December 2004. Completed the requirements for the Master of Science in Entomology at Oklahoma State University, Stillwater, Oklahoma in May 2008.

Experience: Organic Chemistry Teaching Assistant, Department of Chemistry, from August 2002 to December 2004. Field and laboratory research technician from May 2001 to December 2004, and Teaching/Research Assistant from January 2005 to present, Department of Entomology and Plant Pathology, Oklahoma State University.

Professional and Honor Memberships: Entomological Society of America, 2005 to present; Gamma Sigma Delta National Honor Society from 2006 to present; Honor Society of Phi Kappa Phi, 2007 to present; Oklahoma State University Graduate and Professional Student Government representative 2007 to present.

Name: Matthew Paige Smith Date of Degree: May 2008

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: Subterranean Termites of the Oklahoma Tallgrass Prairie Preserve Cross Timbers

Pages in Study: 61 Candidate for the Degree of Master of Science

Major Field: Entomology

Scope and Method of Study: The five objectives of this study were: 1) identify termite species morphologically with molecular verification, 2) delineate colony foraging territories, 3) estimate numbers of foraging termites, 4) compare foraging territory areas, and 5) determine soldier percentages.

Findings and Conclusions: Termites within the Cross Timbers prescribed-burn area were *Reticulitermes flavipes* that forage over areas ranging from 13.5 to 58.5m^2, and contain 59,249 to 422,780 foraging termites. Soldiers comprised 0.53 to 2.08% of the foragers. Termites on the Cross Timbers no-burn area were *R. hageni* that foraged over areas of 18 to 27m^2, with 27,715 to 207,141 foragers. Soldiers comprised 0.76 and 0.96% of foragers. Both prescribed-burn and no-burn sites contained moderately similar termite foraging populations and foraging areas.

ADVISER'S APPROVAL: Bradford M. Kard