SUBTERRANEAN TERMITE (ISOPTERA: RHINOTERMITIDAE)

IMPACT ON PLANT BIOMASS, LITTER AND FEEDING PREFERENCES

ON A TALLGRASS PRAIRIE PRESERVE

By

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Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
May, 2016
SUBTERRANEAN TERMITE (ISOPTERA: RHINOTERMITIDAE)
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ACKNOWLEDGEMENTS

I would also like to thank my committee members Drs. Brad Kard, Jack Dillwith, Mark Payton and Hailin Zhang for all their support. I would especially like to thank Dr. Kard for encouraging me to take on this extensive journey and for the many hours he spent helping me achieve this goal.

I would like to thank God for being my foundation and for blessing me with good health and a wonderful husband. I would like to thank my husband, Matthew, who assisted me on this project and for all his love, support, encouragement and laughter we shared. Together we achieved this awesome goal and will forever be TEAM SMITH!
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Date of Degree: MAY, 2016

Title of Study: SUBTERRANEAN TERMITE (ISOPTERA: RHINOTERMITIDAE) IMPACT ON PLANT BIOMASS, LITTER AND FEEDING PREFERENCES ON A TALLGRASS PRAIRIE PRESERVE

Major Field: ENTOMOLOGY

Scope and Method of Study: There are three objectives in these studies investigating the biology and behavior of subterranean termites on a tallgrass prairie preserve (TGPP). The first study examines the effects of Reticulitermes sp. on plant leaves and stems and root growth in areas with (non-baited) and without (baited) termites. The second study uses mesh bags to determine the influence of subterranean termites on litter loss on the soil surface and sub-surface, in non-baited and baited areas. The final study is a twelve week choice and no-choice termite feeding study using the four predominant grasses of the TGPP.

Findings and Conclusions: Leaf and stem weights showed that above-ground plant biomass within plots containing actively foraging termites was significantly greater compared with plots without termites. However, root biomass was similar within all plots. Results indicate subterranean termite foraging activity within a native tallgrass prairie is beneficial to plant growth. Litter losses between mesh bags located above-ground compared with mesh bags below-ground in both non-baited and baited plots were significant. Litter loss in non-baited plots for above-ground bags was 22.3%, while below-ground bags had a 35.5% loss. Litter bags located above-ground in baited plots showed a 25.0 % loss, while below-ground bags had a 35.7 % loss. There was no significant difference in litter loss between non-baited and baited plot mesh bags. Feeding preferences of the four most predominant TGPP grasses and their roots in the choice and no-choice study were significant. Reticulitermes tibialis preferred switchgrass roots and least preferred little bluestem grass in the choice study. In the no-choice study, big bluestem roots and indiangrass roots were the most preferred food choice and the least preferred were switchgrass root and little bluestem grass.
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CHAPTER I

INTRODUCTION

The Nature Conservancy’s Tallgrass Prairie Preserve (TGPP) located in Osage County, Oklahoma encompasses 15,621 hectares and is the largest tallgrass prairie in the world (Brown et al. 2008). The TGPP is 90% grasslands with the majority being tallgrass (Palmer 2007). The predominant grasses are Indiangrass \([Sorghastrum nutans\ (L.)]\), switchgrass \([Panicum vigatum\ L.]\) big bluestem \([Andropogon gerardii\ Vitman]\), and little bluestem \([Schizachyrium scoparium\ (Michx.)]\) (Palmer 2007). Subterranean termites \((Reticulitermes\ sp.)\) are abundant on the TGPP with an estimated foraging population of 10,357 to 183,495 individuals per colony, and a foraging distance of 19m (62 ft.) (Brown et al. 2008). It is difficult to study termites on the TGPP because of the thick vegetation. The only indication of their presence is during mating flights and occasionally if seen on the soil-surface. To date, there has been no information reported on the effects of \(Reticulitermes\ sp.\) on tallgrass prairie plants and soil in Oklahoma. What they are eating and their effects on plant growth has long been a question among researchers on the TGPP. This current study is a twelve-week choice, no-choice laboratory feeding study using termites and dried big bluestem, little bluestem, indiangrass and switchgrass collected from the TP GG. Although \(Reticulitermes\ sp.\) are not mound builders, termites that build mounds improve plant growth by contributing to increased water availability
and soil nutrient levels. Other species negatively affect plants by feeding on plant roots and stems and are considered to be pests (Abe et al. 2000). To examine the effects of termites on plants, plants and roots where randomly collected and chemically analyzed from areas with and without termites. As foraging activity of Reticulitermes sp. tends to concentrate near the soil-surface (Traniello and Leuthold 2000), surface and sub-surface mesh bags containing organic litter where observed to quantify litter consumption.

Objectives

I. Determine the influence of subterranean termites on plant biomass within the tallgrass habitat in areas with (non-baited) and without (baited) termites.

II. Determine the influence of subterranean termites on litter removal and consumption on the soil surface, and sub-surface.

III. Conduct a twelve-week choice and no-choice termite feeding study using the four predominant grasses on the TGPP.
References Cited


CHAPTER II

REVIEW OF LITERATURE

General Termite Introduction

**Biology and Ecology.** Termites are eusocial insects in the order Isoptera, and generally have three castes: primary reproductives (founding queen and king), workers, and soldiers. Caste members can be either male or female. Alates are winged reproductives (swarmers) that disperse from their parent colony to establish new colonies. For different termite species, flight distances can vary depending on environmental factors such as wind currents and vegetation (Thorne and Forschler 1999). *Reticulitermes virginicus* alates have been captured via aircraft at altitudes up to 914 m (Light 1934). After these nuptial dispersal flights, both female and male alates will break off their wings at a suture line present near the base of the wing and search for a nesting site. Females emit a pheromone that attracts males, and in tandem a single mating pair will locate a warm, moist area within or near wood where they will construct a royal chamber and mate (Thorne and Forschler 1999). In this newly constructed royal chamber, the female initially will lay only 15-30 eggs that gestate for 10 to 50 days. The queen and king will care for and feed the first group of immature termites. The immatures will develop into third instars within one-to-two months, and then assume
nursing duties for a second batch of eggs (Snyder 1935, Ebeling 1975, Su and Scheffrahn 2005). Morphologically, the king only changes slightly from his original form, whereas the queen undergoes physogastry, which is the expansion of her abdomen. This abdominal expansion increases over time as the size of her ovaries increases, and fecundity increases due to the urgent needs of the growing colony (Banks and Snyder 1920, Harris 1961, Lee and Wood 1971). Newly hatched white immatures are termed larvae during their first and second instars. Larvae are soft-bodied with no sclerotization (Thorne and Forschler 1999). During their late third instar, larvae develop into workers, soldiers, or nymphs, and develop sclerotized heads (Harris 1961). Both nymphs and workers can develop into secondary reproductives (neotenics) that stay within the colony. Secondary reproductives derived from nymphs are referred to as nymphoids, and possess wing buds. Those derived from workers are referred to as ergatoids, and do not possess wing buds. Nymphs that become alates will not occur in the colony for three-to-five years, when they will swarm and disperse to start new colonies. Nymphs can also undergo regressive molts to become false workers (pseudergate) that remain with their parent colony (Thorne and Forschler 1999, Jones and Howell 2000, Su and Scheffrahn 2005). A pseudergate can develop into a reproductive or soldier by molting (Lee and Wood 1971).

Workers are the most numerous members and are responsible for foraging for food, nursery maintenance, egg and larval care, building mud tunnels, and grooming nestmates (Lee and Wood 1971, Thorne and Forschler 1999, Potter 2004). In some species, workers help defend the colony by attacking and biting invaders and predators (Prestwich 1984). They also help protect the colony against predators by quickly repairing nest
damage and sealing up flight exit holes. Workers feed soldiers, reproductives, other workers and larvae by trophollaxis, which also is believed to spread pheromones that influence caste development (McMahan 1969). Workers mature within the first year and live up to three years and a colony can live for decades depending on environmental conditions (Potter 2004). A colony with millions of members can consume as much as 0.5 kg (1.1 lb.) of wood per day (Grace 1992).

Soldiers are wingless, eyeless, and sterile, and have enlarged heads equipped with thick sclerotized mandibles that are used to protect nestmates from predators such as ants. They guard any openings occurring within the colony workings and will warn the rest of the colony of danger by creating a ‘stacatto popping’ sound by banging their heads against a substrate (Berenbaum 1995, Potter 2004).

**Nesting and Foraging.** Termites are one of the most important macro-invertebrate decomposers. As soil engineers, they have a great influence on the ecosystem by building structures either above or below ground (Jouquet et al. 2011). Termites can use their saliva, feces, soil and organic material (leaves, grass, animal remains, and woody material) to form mounds, mud tubes, galleries, and nest chambers (Jouquet et al. 2011). Different species of termites build nests that help ensure survival and meet the needs of a growing population. These “biogenic structures” are custom built to maintain constant temperatures, humidity, and protect against predators (Jouquet et al. 2006). There are three different types of nests: subterranean, epigeal, and arboreal (Noirot and Darlington 2000). Subterranean nests are built below ground and consist of enclosed galleries and chambers with few openings (Noirot and Darlington 2000). Lengths of galleries have been reported up to 40 to 50 meters (Greaves 1962, Darlington 1982, Holt and Lepage
Subterranean termites in the genus *Reticulitermes* connect below ground tunnels to above-ground mud tubes (Abe 1987, Shellman-Reeve 1997, Mizumoto and Matsuura 2013). Mud tubes constructed by worker termites provide a safe passage for foraging above ground. Larger, older workers (fourth instar) of *R. fukienensis* were found to be better suited for mud tube construction, while smaller workers and larvae repaired damage to the galleries (Crosland et al. 1998).

An epigeal nest starts out subterranean until the colony reaches a population threshold. Young colonies build nests that have a continuous wall and surrounded by a paraecie, which can be empty or filled with sand (Noirot and Darlington 2000). The paraecie protects the outer wall of the nest from the surrounding soil. As the colony matures the nest will expand upward forming a mound on the soil surface (Noirot and Darlington 2000, Cosarinsky 2011). Depending on the termite species, some internal structures, such as cavities, can be found in the paraecie.

Arboreal nests are built around and attached to tree branches and trunks, as well as on or in man-made structures. Mud tunnels can be found leading from the elevated nest to the soil surface, providing protection to the termites while foraging (Fontes and Milano 2002, Cosarinsky 2005, Merritt and Starr 2010). This above-ground nest provides protection from predators and floods. In some species of *Amitermes*, *Constrictotermes*, and *Procubitermes*, workers build mud ridges on the tree trunk above the nest to divert rain and protect the nest (Noirot and Darlington 2000). Another arboreal nest builder, *Nasutitermes nigriceps*, builds carton nests that are among the largest sizes known, measuring ~2 m in height with a width of ~1 m (Thorne et al. 1996, Flores-Palacios and Ortiz-Pulido 2005).
The gathering and translocation of the soil used to build these various types of nests alters soil properties, affects water infiltration rates, and the diversity of animals, plants and soil microbes (Jouquet et al. 2011). Termites are constantly working regardless of influences the outside environment, because their below-ground nest and tunneling systems create a temperature and humidity-controlled environment that enables them to remain active, even when other soil macro-invertebrates have been eliminated (Wood and Sands 1978, Collins 1983, Jouquet et al. 2011). Different termite genera affect the ecosystem differently based on their feeding habits and the building materials they use (Jones and Eggleton 2000, Brossard et al. 2007, Jouquet et al. 2011). They play a significant role in breaking down cellulosic material and altering the chemicals found in the soil. Depending on the species, they will feed on different types of plant material including those that are living, dead, and decomposing, and they will also feed on organic matter in the soil (Donovan et al. 2001). Termites are able to digest plant material with the aid of symbiotic micro-organisms (La Fage and Nutting 1978), and they adjust their feeding behaviors depending on the quality of the food, seasonal changes, and nutritional needs of a growing colony.

Based on their trophic habits, termites are assigned to one of five different functional groups: wood-feeding, soil-feeding, soil-wood interface-feeding, litter-feeding, or lichen-feeding (Collins 1984, Eggleton et al. 1996, Eggleton et al. 1997, Jones et al. 1998). Wood-feeding termites will tunnel through woody litter to form nesting chambers. *Microcerotermes parvulus* builds its carton nest around the food source, and feeds on encased roots and stems (Wood and Sands 1978, Collins 1981b). Soil-feeding termites ingest mineralized soil and forage throughout the soil-profile and into surface litter.
Because of this type of feeding, mixtures of sand, silt and organic matter are found in their intestines (Alves et al. 2011). Soil-wood interface feeders, feed on soil that is packed within or under logs and woody debris. They will feed on wood that has become extremely decayed and soil-like (Eggleton et al. 1997). Litter-feeding termites assist in decomposition by consuming dead standing or surface woody vegetation, surface leaf-litter, grasses and humus (Wood and Sands 1978). In the southern Guinea savanna, the grass harvesting termite, Trinervitermes geminatus (Wasmann), climbs standing grass tussocks and cuts them into 2-20 mm pieces. These foraging workers will consume some of this grass for energy, deliver pieces back to the nest, and leave the remainder as surface litter (Ohiagu 1979). Lichen feeders consume algae and moss located on tree bark (Eggleton et al. 1997). Regardless of the type of feeding and nesting, termite salivary excretions, feces, and corpses contribute nutrients back into the ecosystem (Jouquet et al. 2011).

**Termites Endemic to Oklahoma.** Five families of termites are found in the United States: Hodotermitidae, Kalotermitidae, Rhinotermitidae, Termitidae, and Termopsidae (Weesner 1965, Thorne et al. 1993). Rhinotermitidae and Termitidae (subterranean termites) are the only families endemic to Oklahoma. Rhinotermitidae species established in Oklahoma are Reticulitermes flavipes (Kollar), Reticulitermes virginicus (Banks), Reticulitermes hageni Banks, and Reticulitermes tibialis Banks. One Termitidae species, Gnathamitermes tubiformans (Buckley), was collected in Tillman County in 1965 (Brown et al. 2004) and Jackson County in 2005 (Smith et al. 2010).

*Reticulitermes flavipes* is considered the most important economic termite pest in the United States (Potter 2004), and its colonies can contain 100,000 to 3,000,000 individuals
that may forage up to 71 m (232 ft.) from the main nest (Grace et al. 1989, Su et al. 1993). Bodies of *R. flavipes, R. virginicus*, and *R. tibialis* alates are dark brown-to-black (Su et al. 2007). *Reticulitermes hageni* alates’ yellowish-brown body and overall length ranging from 7-8 mm including wings, are occasionally misidentified as *Coptotermes formosanus*, which is 12-15 mm-long including wings, due to their similar body color. *Reticulitermes flavipes* alates are ~10-mm-long including wings, which on average are larger than *R. virginicus* alates, whose overall length ranges from 7-8 mm including wings (Potter 2004, Su et al. 2007). *Reticulitermes* wings are white or smokey colored with two longitudinal pigmented veins extending along the front margin, similar to *C. formosanus*. However, *C. formosanus* wing surfaces are covered with minute hairs (Gleason and Koehler 1980).

*Gnathamitermes tubiformans* is a desert termite found on prairies or in semi-arid regions of Arizona and Northern Mexico, New Mexico, Texas and Oklahoma (Allen et al. 1980, Smith et al. 2010). *Gnathamitermes tubiformans* lives in the soil in pastures and grasslands, and is sometimes found under rocks and in cow manure (Smith et al. 2010). Although considered a pasture pest by ranchers due to the amount of vegetation they consume, they are valuable decomposers of organic matter (Mackay et al. 1987). According to Bodine (1975), plots in west Texas free of the desert termites had a 50% increase in ground surface litter. Their food includes the cellulosic material in cow manure and vegetation, including but not limited to the roots and stems of Honey mesquite, Blue grama, and Buffalo grass. This termite encases its food with mud sheets and tubes, constructed at night and after significant rainfall, which provide protection against desiccation and attack from predators (Banks and Snyder 1920, Snyder 1954,
Ueckert et al. 1976, MacKay et al. 1985). Soldiers and workers are identified by their elongated yellowish-orange heads. Soldier mandibles are relatively long and straight, but are curved inward at their tips and have only one tooth on the inner margin of each mandible. Alates are dark brown, measuring more than 13-mm long including wings. They swarm in both spring and summer after rain storms (Snyder 1954, Gleason and Koehler 1980, Nutting 1990).

**Surface Litter.** Surface litter is the accumulation of dead plant materials such as leaves, twigs, stems, and dead wood that has fallen to the ground. The litter can be classified based on each layers’ stage of decomposition (Miura and Matsumoto 1998). Litter decomposition is important for adding nutrients to the soil and supporting soil fauna and microbes. Breakdown of surface litter can be caused by microbial decomposition, leaching, and abiotic and biotic processes (Anderson 1973). Due to their feeding activities, soil animals such as Collembola, Acari, Isopoda, Diplopoda, and earthworms can contribute to litter breakdown (Anderson 1973). Surface litter can be physically broken down by drying, wind, rain, insect activity and mammalian activities (Anderson 1973, Araujo et al. 2012). Annual fires can also destroy surface litter. According to Collins (1981b), annual fires destroyed 31% of total annual litter while termites consumed 24% in Nigeria. Increased temperatures and photodegradation in semi-arid and arid environments have been found to increase the breakdown of litter at the soil-surface (Vossbrinck et al. 1979, Whitford 2002, Pancotto et al. 2005, Gallo et al. 2006, Martinez-Yrizar et al. 2007). Chemical characteristics of litter such as a higher carbon and nutrient content can aid in accelerated decomposition (Perez-Harguindeguy et al. 2000, Wright and Westoby 2002, Vaieretti et al. 2005, Martinez-Yrizar et al. 2007).
However, in semi-arid ecosystems ultraviolet radiation caused 50% of carbon loss in litter. This carbon loss subsequently reduced decomposition of surface litter by 60%, causing an increase in surface litter (Austin and Vivanco 2006).

Soil animals such as termites are effective decomposers of excess surface litter in arid environments; termites in desert ecosystems can consume up to 85% of surface litter (Whitford et al. 1982, Martinez-Yrizar et al. 2007, Dawes 2010). Litter decomposition by soil-dwelling animals can also depend on the type of vegetation that is available and if it is palatable (Anderson 1973, Araujo et al. 2012). Surface litter harvesting can also depend on weather conditions (Park et al. 1993). Excess moisture and temperatures can cause an increase in fungal growth, and more feeding activity by soil animals (Anderson 1973, Araujo et al. 2012). The Western Australian termite, *Drepanotermes tamminensis* (Hill), prefers to harvest surface litter of *Eucalyptus capillosa* after rainy periods when temperatures are between 15 and 25°C (Park et al. 1996). The subterranean desert termites *Gnathamitermes perplexes* (Banks), *G. tubiformans* and *Heterotermes aureus* foraging activities result in litter consumption, soil turnover and nutrient cycling (Nutting et al. 1987, Martinez-Yrizar et al. 2007, Smith et al. 2010). In the plains of the Sonoran Desert of northwestern Mexico, dry conditions did not deter *H. aureus* from consuming the contents of surface litter bags as reported by Martinez-Yrizar et al. (2007). Litter foraging termites in the family Termitidae feed on fungi, grasses, dung, humus, lichen and dead wood (Wood 1978, Noirot 1992, Miura and Matsumoto 1998). The nasute termite *Longipeditermes longipe*, nests underground and forages above-ground for surface litter (Miura and Matsumoto 1998). *L. longipe* workers collect litter only from the lower parts of the forest floor, which is newly decayed, moist, and rich in nitrogen.
Worker termites chew the litter into a food ball and take it back to the nest (Miura and Matsumoto 1998). However, *Macrotermes carbonarius* prefers surface litter that has little decomposition, indicating different food preferences between species (Matsumoto and Abe 1979).

Seasonal foraging patterns, along with nest structure can play a role in food preferences (Collins 1981b). Termites that forage for surface litter risk desiccation, especially during dry seasons. Macrotermiteinae such as genera *Microtermes* and *Ancistrotermes* avoid desiccation by increasing foraging during rainy seasons. During the dry season they feed on fungus combs, which are constructed within (<30 cm in diameter) subterranean nests (Josens 1977, Wood 1978, Collins 1981b). During the dry season, *Ancistrotermes* forages on the soil-surface by building protective mud tubes and mud plastering over tree trunks. Although they will feed on tree bark, they mostly feed on woody litter (Collins 1981b). A grass and wood feeding termite, *Odontotermes pauperans*, constructs subterranean comb nests (~30 cm in diameter) and feeds on grass litter during the dry season. *Macrotermes bellicosus* nests contain combs that are three meters or larger in diameter, which helps provide both protection from the harsh savanna climate while ensuring a plentiful food source. This termite was reported to remove 239 kg ha\(^{-1}\) yr\(^{-1}\) of surface litter (Collins 1981b, a). Macrotermiteinae are the major foragers of soil-surface litter and according to Collins (1981b) they removed 60% of wood litter and 3% leaf litter per annum on the southern Guinea savanna of Nigeria.

**Effects of Termites on Plants.** Termite mounds contain finer particles and more clay, higher levels of nitrogen, more of the exchangeable cations Ca, K, Mg, Na, and contain higher concentrations of organic matter than surrounding soils (Lobry de Bruyn and
Conacher 1990, Lobry de Bruyn and Conacher 1995, Maduakor et al. 1995, Folgarait 1998, Konaté et al. 1999, Jouquet et al. 2006, Brossard et al. 2007). The added nutrients along with added infiltration rates can aid in plant growth and placement of plant communities (King 1977, Woodell and King 1991, Dean et al. 1997, Blomqvist et al. 2000, Holt and Lepage 2000, Folgarait et al. 2002, Jouquet et al. 2004, Jouquet et al. 2006). An example of plant placement by termites is the behavior of the fungus-growing termite *Odontotermes n. pauperans* and its preference for the grass *Imperata cylindrica* (Jouquet et al. 2006). This grass can be found growing on its mounds, while other grasses found within the same savanna do not. This grass was the preferred choice in a feeding study (Konaté 1998, Jouquet et al. 2006). According to Garba et al. (2011), tomato plants grown in soil amended with termite mound material exhibited increased plant growth and fruit production. Nests of fungus-growing termites, Macrotermitinae, found in the African savannah contain nutrients that aid growth of grasses (Jouquet et al. 2005). In a greenhouse study, grasses grown in termite mound soil collected from underground nests exhibited increased growth. Root growth and branching also increased when plants were grown in nest wall material (Jouquet et al. 2005). During the dry season, plants living in the top soil layers of termite mounds had increased water availability and shed less leaves (Konaté et al. 1999). Termite mounds with increased bulk density (1.74 g/cm³ or higher) decreases plant growth because seeds cannot remain lodged within the packed soil constructed by the mound-building termites *Macrotermes bellicosus*, and *Trineritermes geminatus* (Malaka 1977, Holt and Lepage 2000). However, lower bulk density (1.33 g/cm³) proved to grow some vegetation in the mounds of *Amitermes evuncifer* (Holt and Lepage 2000). Vegetation in a humid savanna
environment exhibited growth two-to-three times taller on termite mounds than of those plants not on mounds (Abbadie et al. 1992, Konaté et al. 1999). These mounds provide nutrients, water, and an excellent soil environment that can support vegetation normally found in wet forests (Konaté et al. 1999).

Termite mounds are not the only termite structures that can host plant life. According to Flores-Palacios and Ortiz-Pulido (2005), epiphyte orchids can grow on termite carton trails on trees. The carton nest material is made of saliva, macerated wood and feces, which provides a nutritional substrate for epiphytes (Kofoid 1965, Peaking and Josens 1975, Wood and Sands 1978, Thorne et al. 1996, Eggleton et al. 1997, Flores-Palacios and Ortiz-Pulido 2005). Not only does carton provide nutrition, but also a secure and safe anchor to the tree (Flores-Palacios and Ortiz-Pulido 2005). In a tropical environment, soil conditions can be improved by attracting termites with a food source such as mulch. Mulch provides a barrier on the soil-surface, which helps reduce erosion and soil hardness. The barrier also traps moisture and serves as a habitat for termites. The foraging and tunneling activities of termites increases soil-surface roughness and reduces rain runoff, which increases soil moisture and promotes plant growth (Dawes 2010).

Nitrogen Fixation in Termites

Symbiosis Between Termites and their Gut Microbes. Symbiosis is the nutritional mutualism between a host and a variety of microorganisms. This symbiosis is generally between two organisms, a host such as an animal or plant, and the smaller microorganisms that live within the host (Abe et al. 2000). Termite hindguts contain numerous microorganisms which can include, depending on species, protozoans and
fungi (Eucarya), Archaea and Eubacteria. These microbes and fungi are instrumental in the breakdown of cellulose obtained through the diet and they also play a part in nitrogen (N$_2$) fixation, the metabolism of pyruvate, and the acetogenic reduction of carbon dioxide (CO$_2$) (Breznak et al. 1973, Abe et al. 2000). Fixed nitrogen is incorporated into the termite’s tissues, and is also distributed to the rest of the colony via trophollaxis (Bentley 1984, Waller and La Fage 1987, Curtis and Waller 1998). This process enriches the colonies’ diet with nitrogen and adds nitrogen to the soil in the form of ammonia (NH$_3$) (Schaefer and Whitford 1981, Slaytor and Chappell 1994, Curtis and Waller 1998). In a process called nitrification, ammonia can be converted to nitrite and then nitrate by the soil bacteria Nitrobacter and Nitrosomas. The resulting nitrate, nitrite and ammonia are taken up by plant roots in the soil (NPAP 2008).

**History of the Study of Nitrogen Fixation in Termites.** Termites have a reputation for their destructive behavior, costing 20 billion U.S. dollars annually in structural damages, repairs, and treatments, and are rarely thought of as a beneficial insect. However, termites are ecologically important, contributing to the increase in soil aeration and drainage, and changing the complex carbohydrates of cellulose-containing material to simple carbon compounds. Their gut symbionts are able to fix atmospheric nitrogen, which fertilizes and nourishes the soil by adding nitrogen compounds (Breznak et al. 1973).

Cleveland (1925) concluded that termites utilize nitrogen fixation to survive on a low nitrogen diet. He reported that when protozoa were removed from the termite gut by starvation or oxygenation, termites could no longer live on a diet of wood or cellulose. When the protozoa were reintroduced into *Reticulitermes* and *Termopsis*, the ability to
digest cellulose was restored (Cleveland 1925). Further laboratory and field studies conducted on *Termopsis* confirmed Cleveland’s findings. The acetylene-ethylene assay uses a gas chromatograph to measure nitrogenase activity by measuring the reduction of acetylene to ethylene (Hardy et al. 1968, Breznak et al. 1973). This method was made possible by the research of Dilworth (1966), who found that nitrogenase would reduce acetylene to ethylene in a manner similar to the reduction of N\textsubscript{2} to NH\textsubscript{3}. Specimens of *Cryptotermes brevis* (Walker), *Zootermopsis* sp., *C. formosanus* Shiraki workers, and *R. flavipes* (Kollar) had their guts removed and assayed. The reduction of acetylene to ethylene was then measured (Mertins et al. 1973). The termite bodies alone did not show any activity, suggesting microorganisms in the hindgut were responsible for this nitrogen fixation activity (Mertins et al. 1973). Specimens of *C. formosanus* Shiraki were assayed for acetylene reducing activity by feeding them treated filter paper. One group of termites was fed filter paper impregnated with distilled water and another group was fed filter paper impregnated with a nitrogen source. Termites that fed on the nitrogen impregnated filter paper showed a decrease in acetylene reducing activity compared with a laboratory colony feeding on wood, while termites that fed on filter paper containing distilled water showed a 10-fold increase in activity compared with the laboratory colony (Mertins et al. 1973). Termites that fed on paper containing antibiotics directed towards eliminating bacteria only, showed a complete loss of acetylene reduction. Protozoa in this experiment and in similar experiments were not initially affected, but disappeared after 20 days or longer. These experiments indicate that prokaryotes in the gut are responsible for nitrogen fixation and that bacteria are vital to the survival of the protozoa (Breznak et al. 1973, Mertins et al. 1973).
All termites do not have protozoa in their hindguts. Higher termites (Termitidae) contain bacteria but no protozoa, while lower termites like those in the families Rhinotermitidae and Kalotermitidae contain both bacteria and protozoa in the gut. Nitrogen fixation has been confirmed in both higher and lower termites (French et al. 1976). *Coptotermes formosanus* Shiraki fed filter paper impregnated with ammonium \((\text{NH}_4^+)\) and then transferred to filter paper with no \(\text{NH}_4^+\) showed a rapid increase in acetylene reduction in a short amount of time (Breznak et al. 1973). A second group of termites allowed to continue feeding on the \(\text{NH}_4^+\) filter paper continued to have low levels of acetylene reduction. This data shows how this species’ nitrogen fixation system is quick and efficient, except in soldiers, which is not always the case with certain species or castes (Breznak et al. 1973). The Drywood termite, *Kalotermes minor*, showed reduction of acetylene in workers, but relatively lower rates of acetylene reduction in both reproductives and soldiers. This lower rate of reduction might be due to feeding of these caste members via trophallaxis. Low acetylene reduction activity was also observed between different termite worker groups and might be due to the different ages of the termites (Benemann 1973). In contrast, acetylene reducing activity in larvae of *C. formosansus* exhibited 200-fold higher rates. At this high fixation rate, a colony could double their nitrogen content in a year (Breznak 1975, Potrikus and Breznak 1977). Species body size can also affect different reduction rates of acetylene activity. After a four-hour assay at 25°C, the relatively small size termite, *Coptotermes lacteu* (Froggatt), had the lowest nitrogenase activity of 0.03 nmol \(\text{C}_2\text{H}_4\)/h, whereas *Nasutitermes exitiosus* (Hill) had a higher activity at 0.11 nmol \(\text{C}_2\text{H}_4\)/h (French et al. 1976). The largest size species, *Mastotermes darwiniensis* Froggatt, had considerably more acetylene reduction
at 5.5 nmol C$_2$H$_4$/h. Additional data has shown that termite workers of approximately the same size also have similar nitrogen fixing abilities (French et al. 1976). Other insects have been tested for acetylene reduction, including Pea aphids, German cockroaches, Milkweed bugs, American cockroaches, Lesser Grain Borers, Drugstore Beetles, Brown-banded cockroaches, and Yellow mealworms, but none exhibited acetylene reduction (Breznak et al. 1973).

**Molecular Techniques.** Whereas identification of termites to species can be difficult due to overlapping morphological measurements between the soldier caste of different species, and as alates are only available during their swarming season(s), molecular techniques can identify termites to species, regardless of their caste. Molecular techniques can also be used to determine genetic variation and phylogenetics of a species. Sequencing a portion of the mitochondrial DNA (mtDNA) 16S rRNA gene was done to determine genetic variation of *Reticulitermes* sp. throughout the United States of America (USA) and world-wide (Austin et al. 2004a, Austin et al. 2004b, Austin et al. 2005, Austin et al. 2006). *Reticulitermes* sp. throughout the USA have been identified utilizing the 16S gene (McKern et al. 2006, Szalanski et al. 2006, King et al. 2007). Description and validation of *R. malletei* collected from Delaware, Georgia, Maryland, North Carolina and South Carolina, as well as *R. okanaganensis* collected from Idaho and British Columbia was accomplished through 16S gene sequencing (Szalanski et al. 2006, Austin et al. 2007). Additionally, genetic variations between *Reticulitermes* sp. from Asia, Europe and North America have been determined using DNA sequencing of the mtDNA cytochrome oxidase II (COII) gene. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using the COII and 16S genes, has also
been utilized to identify *Reticulitermes* to species (Szalanski et al. 2003). Foster et al (2004) identified *R. flavipes* using a region of AT-rich mtDNA. PCR has been instrumental in helping us identify old and new species of termites, identify their symbiotic protists and bacteria, and help us understand termite populations and geographic distribution (Austin et al. 2004b, Sato et al. 2014).
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CHAPTER III

SUBTERRANEAN TERMITE (ISOPTERA: RHINOTERMITIDAE) INFLUENCE ON PLANT BIOMASS ON A NATIVE TALLGRASS PRAIRIE
ABSTRACT

Recent studies investigating subterranean termite ecology have been conducted on The Nature Conservancy’s Joseph H. Williams Tallgrass Prairie Preserve near Pawhuska, northeast Oklahoma. None of these studies examined effects of termites on plant growth. This study examined the effects of *Reticulitermes* sp. on plant leaves and stems and root growth. Four field-sites, each consisting of four blocks of land with each block further sub-divided into two plots, were established. Using baits, termites were removed from one plot in each block. From all plots with (non-baited) or without (baited) termites, a total of 192 blade and stem samples and 192 root samples were collected and differences in their biomass measured. Leaf and stem weights showed that above-ground plant biomass within plots containing actively foraging termites was significantly greater compared with plots without termites. However, root biomass was similar within all plots. Results indicate subterranean termite foraging activity within a native tallgrass prairie is beneficial to plant growth. The increase in growth may be attributed to termites chewing on the roots, stimulating more overall growth. The root biomass shows no difference because the increased growth is negated by the removal of root tissue by the foraging termites.

KEY WORDS  *Reticulitermes*, Rhinotermitidae, subterranean termites, tallgrass prairie
Introduction

**Biology and Ecology.** Termites are eusocial insects in the order Isoptera, and generally have three castes: primary reproductives (founding queen and king), workers, and soldiers. Caste members can be either male or female. Alates are winged reproductives (swarmers) that disperse from their parent colony to establish new colonies. For different termite species, flight distances can vary depending on environmental factors such as wind currents and vegetation (Thorne and Forschler 1999).

*Reticulitermes virginicus* alates have been captured via aircraft at altitudes up to 914 m (Light 1934). After these nuptial dispersal flights, both female and male alates will break off their wings at a suture line present near the base of the wing and search for a nesting site. Females emit a pheromone that attracts males, and in tandem a single mating pair will locate a warm, moist area within or near wood where they will construct a royal chamber and mate (Thorne and Forschler 1999). In this newly constructed royal chamber, the female initially will lay only 15-30 eggs that gestate for 10 to 50 days. The queen and king will care for and feed the first group of immature termites. The immatures will develop into third instars within one-to-two months, and then assume nursing duties for a second batch of eggs (Snyder 1935, Ebeling 1975, Su and Scheffrahn 2005). Morphologically, the king only changes slightly from his original form, whereas the queen undergoes physogastry, which is the expansion of her abdomen. This abdominal expansion increases over time as the size of her ovaries increases, and fecundity increases due to the urgent needs of the growing colony (Banks and Snyder 1920, Harris 1961, Lee and Wood 1971). Newly hatched white immatures are termed larvae during their first and second instars. Larvae are soft-bodied with no sclerotization
During their late third instar, larvae develop into workers, soldiers, or nymphs, and develop sclerotized heads (Harris 1961). Both nymphs and workers can develop into secondary reproductives (neotenics) that stay within the colony. Secondary reproductives derived from nymphs are referred to as nymphoids, and possess wing buds. Those derived from workers are referred to as ergatoids, and do not possess wing buds. Nymphs that become alates will not occur in the colony for three-to-five years, when they will swarm and disperse to start new colonies. Nymphs can also undergo regressive molts to become false workers (pseudergate) that remain with their parent colony (Thorne and Forschler 1999, Jones and Howell 2000, Su and Scheffrahn 2005). A pseudergate can develop into a reproductive or soldier by molting (Lee and Wood 1971).

Workers are the most numerous members and are responsible for foraging for food, nursery maintenance, egg and larval care, building mud tunnels, and grooming nestmates (Lee and Wood 1971, Thorne and Forschler 1999, Potter 2004). In some species, workers help defend the colony by attacking and biting invaders and predators (Prestwich 1984). They also help protect the colony against predators by quickly repairing nest damage and sealing up flight exit holes. Workers feed soldiers, reproductives, other workers and larvae by trophallaxis, which also is believed to spread pheromones that influence caste development (McMahan 1969). Workers mature within the first year and live up to three years and a colony can live for decades depending on environmental conditions (Potter 2004). A colony with millions of members can consume as much as 0.5 kg (1.1 lb.) of wood per day (Grace 1992).
Soldiers are wingless, eyeless, and sterile, and have enlarged heads equipped with thick sclerotized mandibles that are used to protect nestmates from predators such as ants. They guard any openings occurring within the colony workings and will warn the rest of the colony of danger by creating a ‘stacatto popping’ sound by banging their heads against a substrate (Berenbaum 1995, Potter 2004).

**Nesting and Foraging.** Termites are one of the most important macro-invertebrate decomposers. As soil engineers, they have a great influence on the ecosystem by building structures either above or below ground (Jouquet et al. 2011). Termites may use their saliva, feces, soil and organic material (leaves, grass, animal remains, and woody material) to form mounds, mud tubes, galleries, and nest chambers (Jouquet et al. 2011). Subterranean nests are built below ground and consist of enclosed galleries and chambers with few opening (Noirot and Darlington 2000). Subterranean termites in the genus *Reticulitermes* connect below ground tunnels to above-ground mud tubes (Abe 1987, Shellman-Reeve 1997, Mizumoto and Matsuura 2013). Mud tubes constructed by worker termites provide a safe passage-way for foraging above and below ground. Soil transported and used to build these various types of nests, alters soil properties, affects water infiltration rates, as well as diversity of animals, plants and soil microbes (Jouquet et al. 2011). Termites play a significant role in breaking down cellulosic material and altering chemicals found in the soil. They adjust their feeding behaviors depending on the quality of the food, seasonal changes, and nutritional needs of a growing colony (Collins 1984, Eggleton et al. 1996, Eggleton et al. 1997, Jones et al. 1998).

**Effects of Termites on Plants.** Soil obtained from termite mounds contains fine particles, exchangeable cations of Ca\(^{++}\), K\(^{+}\), Mg\(^{2+}\) and Na\(^{+}\) and contain higher

**Nature Conservancy’s Joseph H. Williams Tallgrass Prairie Preserve (TGPP).**

The Nature Conservancy’s Joseph H. Williams Tallgrass Prairie Preserve (TGPP) in Osage County, 18.5-km (11.5 mi.) north of the town of Pawhuska in northeast Oklahoma (Brown et al. 2009). It is 15,659 hectares (≈ 39,000 acres) consisting mainly of native tallgrass prairie and is home to 2500 bison. The prairie’s most predominant grasses are big bluestem (*Andropogon gerardii* Vitman), switchgrass (*Panicum virgatum* L.) and indiangrass (*Sorghastrum nutans* L.) (Hurt 1999, Brown et al. 2009).

This current study evaluates how the subterranean termites (*Reticulitermes* sp.) affect plant growth in a native tallgrass prairie in a humid subtropical climate. Results provide information on the biomass of plants and their roots in areas with (non-baited) and without (baited) termites. A chemical analysis of the plants and roots collected will also provide information on if the presences of termites add elements to the soil that aid in plant growth.
Materials and Methods

Field Sites and Plots. There are four rectangular field sites, each measuring 130.0 × 50.0 m (426.5 × 164.0 ft.) located on The Nature Conservancy’s Joseph H. Williams Tallgrass Prairie Preserve (TGPP) in Osage County, 18.5-km (11.5 mi.) north of the town of Pawhuska in northeast Oklahoma (Brown et al. 2009). Each field site consists of four rectangular blocks of land, each measuring 50.0 × 10.0 m (164.04 × 32.81 ft.) (Fig. 3.1). Each block consists of two plots, each measuring 10.0 × 10.0 m (32.81 × 32.81 ft.) located at each of the four corners and marked with a 0.5-m (1.6 ft.) wooden stake. Each plot is separated by a 30.0 × 30.0 m (98.4 × 98.4 ft.) buffer zone (Fig. 3.1). Termites on the TGPP are known to have a maximum linear foraging distance of 19.0 m (62.0 ft.) (Brown et al. 2008). To ensure termites from one plot are not overlapping into adjacent plots, buffer zones are greater than the known maximum foraging distances.

Bait Systems. In each block within each field-site, one of two plots per block was randomly selected for termite elimination using baiting systems. Field-sites-1 (FS1) and 4 (FS4) bait plots each contain ten Sentricon® Stations with Recruit™ IV Baitubes containing noviflumuron (Dow AgroSciences LLC., Indianapolis, IN). Field-sites-2 (FS2) and -3 (FS3) bait plots each contain ten Advance® Termite bait stations with bait cartridges containing diflubenzuron (Fig. 3.2A). Each bait station was covered with a 2.4 L (2.5 qt.) inverted metal bucket, and stabilized with a standard building brick (Fig. 3.3A,B). The bucket protects the plastic bait station from melting during TGPP management prescribed burns, and aids in locating the bait station in the tall grass. All termite bait stations were maintained regularly and checked for termite activity.
**Monitoring Devices.** Rectangular pine (*Pinus* sp.) soil-surface ground-boards (SSGB) measuring 30.5 × 15.2 × 2.5 cm (12.0 × 6.0 × 1.0 in.) were used to monitor termite activity. Vegetation was removed and each board placed directly on the bare mineral soil surface. A standard building brick was placed on each SSGB to hold it in place and ensure solid contact with the soil surface (Fig. 3.4) (Brown et al. 2004). In bait plots, two SSGBs were placed at 3.3-m (10.8 ft.) intervals between the center top and bottom stations (Fig. 3.2B). In non-baited control plots, four SSGB were placed at 4.7-m (15.4 ft.) intervals, two on each diagonal between opposite corners (Fig. 3.5). The SSGBs remained in the non-baited plots only until the presence of termites was confirmed, and subsequently removed to avoid providing an alternative food source that could affect the amount of feeding on plants. However, the SSGBs did remain in bait plots to evaluate the continued absence of termites.

**Determination of Plant Biomass.** A total of 384 separate samples, 192 above-ground plant leaf and stem groups, and 192 root groups, were collected from all four research sites during three separate collection events. Each collection consisted of 16 above-ground and 16 root samples per site (32 samples per site). Two above-ground and two root samples were randomly collected from random locations in each plot in all four sites, resulting in the 32 samples per site. Samples (leaves and stems; roots) were collected from within a circular quadrat measuring 0.25 m² (387.5 in²). All above-ground plant biomass within the quadrat boundary was cut off at ground level using steel-bladed shears, and placed into a labeled brown paper bag (Wilson 2009). In the laboratory, each above-ground plant sample was removed from the bag, weighed ‘wet’ and returned to the labeled bag. A root sample from within the quadrat was obtained using a 10.2-cm
diameter × 20.3-cm height (4.0 × 8.0 in.) (1659 cm³) soil-auger, and placed in a labeled brown paper bag. Each root sample was removed from the bag and thoroughly rinsed with water to remove soil, blotted dry, and returned to the labeled bag. Each bag of plant material was oven-dried at 80°C (176°F) for 24 hours, removed, and a dry weight obtained. A portion (0.15 g) of each dried sample was submitted to the Oklahoma State University Soil, Water and Forage Analytical Laboratory for carbon and nitrogen analysis. These samples were dried at 85°C overnight and pulverized to pass through a 1-mm screen. Moisture content of each plant sample was determined by drying the pulverized sample at 105°C. Total nitrogen (TN) and carbon were determined using a dry combustion Carbon/Nitrogen Analyzer (LECO Truspec, NFTA, 1993). Mineral contents of the samples were analyzed by a Spectro CirOs ICP following wet digestion (Undersander et al. 1993).

**Statistics.** Analyses were performed with SAS Version 9.4 (SAS Institute, Cary, NC). Analysis of variance (PROC MIXED) was used to assess the effect of treatment and time. A repeated measures model was utilized in a randomized block design with site as block and time as the repeated factor. An autoregressive period 1 covariance structure was used to model the intra-site variation across time. When interactions of treatment and time are not significant, main effect means and standard errors are reported.

**Results and Discussion**

A total of 384 separate samples, 192 above-ground plant leaf and stem groups, and 192 root groups, were collected from all four research sites during three separate collection events (Table 3.1). There were significant differences in the above-ground plant biomass
between the baited and non-baited plots ($P = 0.0262$) (Fig. 3.6). This suggests that foraging termites are having a positive effect on plant growth. Research conducted in other countries on termite mounds have reported increased plant growth due to termite tunneling and mound building (King 1977, Woodell and King 1991, Dean et al. 1997, Blomqvist et al. 2000, Holt and Lepage 2000, Folgarait et al. 2002, Jouquet et al. 2004, Jouquet et al. 2006). Such construction activities are believed to affect increased chemical nutrition, organic matter and water infiltration in soil (Flores-Palacios and Ortiz-Pulido 2005, Jouquet et al. 2005, Garba et al. 2011). During this study, Osage County Oklahoma was experiencing extreme drought conditions, which would have forced termites to tunnel deeper into the soil looking for moisture. This deeper tunneling activity may have increased water infiltration to deeper roots, as well as delivering soil nutrients to environmentally stressed plants. There was no significant difference in root biomass between baited and non-baited (control) plots. The increase in growth may be attributed to termites chewing on the roots, stimulating more overall growth. The root biomass shows no difference because the increased growth is negated by the removal of root tissue by the foraging termites.

In the laboratory study to determine which of the four most predominant TGPP grasses termites would prefer to eat, *Reticulitermes tibialis* preferred roots over grass leaves and stems in both choice and no-choice tests. However, they did prefer leaves and stems of certain grass species over roots of other grasses. Termites were observed feeding within the stems of big bluestem and indiangrass. If this occurs in natural grassy fields, it is possible that our plant biomass measurements may have been even more significant
because we did not measure feeding within stems. Further research is needed to evaluate this hypothesis.
Fig. 3.1. Each of four field sites consists of four blocks of land, each with two plots. One plot was randomly selected for termite bait installation (red). Control plots (green) are non-baited. Yellow area is the buffer zone to reduce the possibility of termites from one colony being present in adjacent plots.
Fig. 3.2. Baited plot with two termite monitors. (A) Oval represents termite bait station. (B) Rectangles are soil-surface ground-board.
Fig. 3.3. (A) In-ground bait station (top view); (B) Inverted metal bucket placed over bait station and secured with brick.
Fig. 3.4. Soil-surface ground-board (top view).
Fig. 3.5. Non-baited control plot with soil-surface ground-boards (top view).
<table>
<thead>
<tr>
<th>Block/Plot</th>
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Four blocks per site with each block containing two plots (one baited and one non-baited)
Two plant samples with their roots were collected from each plot
B= Baited Plot (no termites)  N=Non-Baited Plot (termites present)
Above-ground plant biomass comparison of non-baited plots and baited plots ($P = 0.0262$).
Acknowledgements

The author would like to thank Bob Hamilton and The Nature Conservatory’s Joseph H. Williams Tallgrass Prairie Preserve for allowing us to work on the prairie. Thanks to Lisa Coburn and Dr. Richard Grantham for their assistance in locating ATVs and other valuable equipment. Thanks to Lucus Pierce and Anndrea Navesky for their assistance in setting up the research field sites.
References Cited


CHAPTER IV

SUBTERRANEAN TERMITE (ISOPTERA: RHINOTERMITIDAE) SOIL-
SURFACE AND SUB-SURFACE LITTER REMOVAL AND CONSUMPTION ON
A NATIVE TALLGRASS PRAIRIE
ABSTRACT

The role of the subterranean termites (*Reticulitermes* sp.) on litter consumption and removal on The Nature Conservancy’s Joseph H. Williams Tallgrass Prairie Preserve (TGPP) in northeast Oklahoma was evaluated. Four field sites, each consisting of four blocks of land with each sub-divided into two plots, were established. Using in-ground baiting systems, termites were eliminated from one plot in each block. A total of sixty-four stainless mesh bags, each containing 100g of a mixture of prairie plant litter, were placed on the soil-surface (32 bags) and another 32 bags were buried 15-cm deep. Mesh bags were randomly distributed among plots containing foraging termites (non-baited) and plots without termites (baited) and evaluated after 12 months. Litter loss for above-ground bags in non-baited plots was 22.3%, whereas loss in baited plots was 25% (w/w). Litter loss for below-ground bags in non-baited plots was 35.5%, whereas loss in baited plots 35.7% (w:w). There were no significant differences in litter loss in non-baited compared with baited plots. However, there was a significant difference in litter loss in above-ground bags compared with below-ground bags.

KEY WORDS  litter, *Reticulitermes*, Rhinotermitidae tallgrass prairie, termite foraging
Introduction

Surface litter is the accumulation of dead plant materials such as leaves, twigs, stems, and dead wood that have fallen to the ground. The litter can be described in different layers that are in different stages of decomposition (Miura and Matsumoto 1998). Litter decomposition is important for adding nutrients to the soil and supporting-soil fauna and microbes. Breakdown of surface litter can be caused by microbial decomposition, leaching, and abiotic and biotic processes (Anderson 1973). Surface litter can be physically broken down by drying, wind, rain, insects and mammal activities (Anderson 1973, Araujo et al. 2012). Annual fires can also destroy surface litter, as well as termites. According to Collins, annual fire destroyed 31% of total annual litter while termites consumed 24% in Nigeria (1981). Termites in families Termitidae and Macrotermiteidae live in semi-arid and arid environments and are major forgers of surface litter. Litter foraging termites in desert ecosystems can consume up to 85% of surface litter (Whitford et al. 1982, Martinez-Yrizar et al. 2007, Dawes 2010). The objective of this study was to examine the possibility that Reticulitermes sp. could be foraging surface litter in a tallgrass prairie habitat. Although unaware of any reports of such behavior from this species on a tallgrass habitat in the United States, we have observed this termite on the soil-surface beneath the thick vegetative layer on the TGPP in Oklahoma. In this current study, litter bags are placed on and below the soil-surface in plots with (non-baited) and without (baited) termites. Information obtained from this study will give a better understanding of foraging habits of Reticulitermes sp. in a tallgrass habitat.
Materials and Methods

Field Sites and Plots. There are four rectangular field-sites each measuring 130.0 × 50.0 m (426.51 × 164.04 ft.) (Fig. 4.1) located on The Nature Conservancy’s Joseph H. Williams Tallgrass Prairie Preserve (TGPP) in Osage County, 18.5-km (11.5 mi.) north of the town of Pawhuska in northeast Oklahoma (Brown et al. 2009). Each field-site consists of four rectangular blocks of land, each measuring 50.0 × 10.0 m (164.04 × 32.81 ft.) (Fig. 4.1). Each block consists of two plots, each measuring 10.0 × 10.0 m (32.81 × 32.81 ft.) located at each of the four corners and marked with a 0.5-m (1.6 ft.) wooden stake. Each plot is separated by a 30.0 × 30.0 m (98.43 × 98.43 ft.) buffer zone (Fig. 4.1). Termites on the TGPP are known to have a maximum linear foraging distance of 19.0 m (62.0 ft.) (Brown et al. 2008). These buffer zones are greater than the known foraging distance to ensure termites from one plot are not overlapping into adjacent plots.

Bait Systems. In each block within each field-site, one of two plots per block was randomly selected for termite elimination using termiticides baiting systems. Field-site-1 (FS1) and field-site-4 (FS4) baited plots each contain ten Sentricon® Stations with Recruit™ IV Baitubes containing noviflumuron (Dow AgroSciences LLC., Indianapolis, IN). Field-site-2 (FS2) and field-site-3 (FS3) treated plots each contain ten Advance® Termite Bait Stations with termite bait cartridges containing diflubenzuron (Fig. 4.2A). Each bait station was covered with a 2.4 L (2.5 qt.) inverted metal bucket, and secured with a standard building brick (Fig. 4.3A,B). The bucket protects the bait station from melting during TGPP management prescribed burns, and aids in locating the bait station in the tall grass. All termite bait stations were maintained regularly and checked for termite activity.
**Monitoring Devices.** Rectangular soil-surface ground-boards (SSGB) made of pine measuring 30.5 × 15.2 × 2.5 cm (12.0 × 6.0 × 1.0 in.) were used to monitor termite activity. Vegetation was removed and each board was placed directly on the bare mineral soil surface. A standard building brick was placed on each SSGB to hold it in place and ensure solid contact with the soil surface (Fig. 4.4) (Brown et al. 2004). In baited plots, two SSGB were placed at 3.3-m (10.8 ft.) intervals between the center top and bottom stations (Fig. 4.2B). In non-baited plots, four SSGB were placed at 4.7-m (15.4 ft.) intervals, two on each diagonal between opposite corners (Fig. 4.5). The SSGB remained in the non-baited plots only until the presence of termites was confirmed, and remained in baited plots to assure the continued absence of termites.

**Litter Consumption and Removal at Soil-Surface and Sub-Surface.** Random above-ground standing plant material and surface litter were collected on the TGPP and cut into 7.62-cm-long pieces (3.0 in.), and placed in a drying oven at 80°C for 24 hours. Sixty-four mesh bags, each measuring 30.0 × 30.0 cm (11.8 × 11.8 in.), were constructed from 27-gauge, 3.0 mm (0.12 in.) square aperture mesh hardware cloth, and filled with 100 g of oven-dried plant material. The aperture size was chosen to allow entry of termites but exclude larger soil-dwelling animals. In both baited and non-baited plots, two mesh bags were randomly placed, one on the soil-surface (Fig. 4.6A) and the other 15.0 cm (6.0 in.) below the soil-surface (Fig. 4.6B) in a different location. For the soil-surface bags, vegetation was removed and one mesh bag was placed directly on the bare mineral soil surface, and covered with a 40.6 × 40.6 cm (16.0 × 16.0 in.) concrete garden paver, to protect the bag during prescribed burns and naturally occurring fires. All bags were removed after one year and the contents of each individual bag placed in a #80
sieve, rinsed with water to remove loose soil, and blotted dry with paper towels, leaving only the original plant material. The plant material was placed in a drying oven at 80°C (176°F) for 24 hours, and a dry weight obtained.

**Statistics.** Statistical analyses were performed with SAS Version 9.4 (SAS Institute, Cary, NC). Analysis of variance (PROC MIXED) was used to assess the effect of treatment and ground. A split-plot arrangement in a randomized complete block design was utilized. Treatment was the main unit factor, and ground was the split unit factor. Factor level means and standard errors are reported, and simple effects are compared with protected pairwise comparisons.

**Results and Discussion**

There was a significant difference in litter loss between mesh bags located above-ground and mesh bags below-ground in both non-baited and baited plots (Fig 4.7). Litter loss in non-baited plots for above-ground bags showed a 22.3% loss, while below-ground bags had a 35.5% loss. Litter bags located above-ground in baited plots showed a 25.0% loss, while below-ground bags had a 35.7% loss. However, there was no significant difference in litter loss between non-baited and baited plot mesh bags. No termite activity or signs of termites in or around the mesh bags were observed. During the time of this research project (2012-2013) Oklahoma’s Osage County experienced ten months of extreme drought (United States Drought Monitor 2013). This could have caused the termites to avoid the soil-surface and retreat deeper into the soil-surface resulting in no physical contact with the mesh bags. Brief periods of rain throughout the year moistened the below-ground bags enough to promote fungal mycelium which would aid in decomposition and weight loss. The garden pavers covering the above-ground bags
protected the bags from direct sun light, dry winds and prescribed burns. Between April and September, temperatures ranged from 32.2° to 43.3° C (90° to 110° F) and would delay decomposition. Although termites were not observed around the bags, other macroinvertebrates were found outside the above-ground mesh bags and may have also affected the presence of termites. Ants were the most abundant termite predator found around the above-ground mesh bags in both baited and non-baited plots. Acrobat Ants (Crematogaster sp.) and Citronella Ants (Acanthomyops sp.) were sometimes found nesting around the parameters of the garden paver located on top of the mesh bag. Other insects observed were the American Cockroach (Periplaneta americana) and beetles (Scarites sp.), which were located under the mesh bags. Other arthropods such as the Black Widow spiders (Latrodectus mactans) and centipedes, found refuge under the edges of the garden paver.
Fig. 4.1. Each field site consists of four blocks of land, each with two plots. One plot is randomly chosen to be baited (red) for termites and the other plot is non-baited (green). Yellow area indicates buffer zone to keep termites from foraging from plot to plot.
Fig. 4.2. Baited plot with two termite monitors.
(A) Oval represents termite bait station. (B) Rectangles are soil-surface ground-broad.
Fig. 4.3. (A) In-ground bait station (top view), (B) Inverted metal bucket placed over bait station and secured with brick.
Fig. 4.4. Soil-surface ground-board (top view).
Fig. 4.5. Non-baited control plot with soil-surface ground-boards (top view).
Fig. 4.6. Views of mesh bags: (A) soil-surface; (B) below soil-surface.
**Fig. 4.7.** Final dry weight of above-ground and below-ground mesh bags in baited (B) and non-baited (NB) plots. Different letters indicate significant differences (α=0.05).
Acknowledgements

The author would like to thank Bob Hamilton and The Nature Conservatory’s Joseph H. Williams Tallgrass Prairie Preserve for allowing us to work on the prairie. Thanks to Lisa Coburn and Dr. Richard Grantham for their assistance in locating ATVs and other valuable equipment. Thanks to Lucus Pierce and Anndrea Navesky for their assistance in setting up the research field sites.
References Cited


CHAPTER V

FEEDING PREFERENCES OF *RETICULITERMES TIBIALIS* (ISOPTERA: RHINOTERMITIDAE) ON FOUR PREDOMINENT GRASSES ON THE JOSEPH H. WILLIAMS TALLGRASS PRAIRIE PRESERVE.
ABSTRACT

Termite researchers on The Nature Conservancy’s Joseph H. Williams Tallgrass Prairie Preserve (TGPP) in northeast Oklahoma have long speculated on the nutritional resources used by termites living within the soil. In this twelve-week choice and no-choice feeding study, subterranean termites (*Reticulitermes tibialis*) were fed a stem-and-leaf mixture, and roots, of the four predominant grasses found on the TGPP: Indiangrass [*Sorghastrum nutans* (L.)], switchgrass (*Panicum vigatum* L.) big bluestem (*Andropogon gerardii* Vitman), and little bluestem [*Schizachyrium scoparium* (Michx.)], in a laboratory setting. Results showed that termites would eat all of these food choices but preferred roots over leaves and stems. In the choice tests, termites preferred switchgrass roots. In the no-choice test, indiangrass root and big bluestem roots were preferred.

**KEY WORDS**  big bluestem, indiangrass, little bluestem, *Reticulitermes*, Rhinotermitidae, subterranean termites, switchgrass, tallgrass prairie
Introduction

Termites are constantly working regardless of the outside environment. Their below-ground nest and tunneling systems create a temperature- and humidity-controlled environment that enables them to keep working when other soil macroinvertebrates have been eliminated due to inhospitable conditions (Wood and Sands 1978; Collins 1983; Jouquet et al. 2011). Different termite genera affect their ecosystem differently based on their feeding habits and building materials they use (Jones and Eggleton 2000; Brossard et al. 2007; Jouquet et al. 2011). They play a significant role in breaking down cellulose material and altering the chemical composition in the soil. Depending on species, they will feed on different levels of plant material including living plants, dead or decomposing plants, and other organic materials in soil (Donovan et al. 2001). Termites are able to digest plant material with the aid of internal symbiotic micro-organisms (La Fage and Nutting 1978). They adjust their feeding behaviors depending on the quality of the food, seasonal changes, and nutritional needs of a growing colony. In this study, we investigated what subterranean termites (Rhinotermitidae: Reticulitermes) are eating on the The Nature Conservancy’s Joseph H. Williams Tallgrass Prairie Preserve (TGPP) in northeastern Oklahoma. Results will provide new information on the feeding habits of Reticulitermes sp. within a tallgrass prairie ecosystem.

Materials and Methods

Termite Feeding. Three groups of Reticulitermes tibialis were obtained from the TGPP and used in 12-week choice and no-choice feeding studies. Some studies used roots of big bluestem (BBR), little bluestem (LBR), indiangrass (INR), or switchgrass (SWR). Additional studies used a near-homogeneous mixture of stems and leaves of big
bluestem (BBG), little bluestem (LBG), indiangrass (ING) or switchgrass (SWG) that were grown in a greenhouse. Termites were maintained in the laboratory in three 38-L (10 gal) galvanized steel garbage cans with lids. The laboratory environment was maintained at 22°C (71.6°F) and 60 percent relative humidity with low-light. However, relative humidity within the garbage can micro-environment ≥ 95%. Termites were provisioned with Monterey pine (Pinus radiata) boards measuring 30.5 × 15.2 × 2.5 cm (12.0 × 6.0 × 1.0 in.) and supplied with water (Kard et al. 2007). Roots of each plant species were cut from the plant, thoroughly washed with tap water, and blotted dry with a paper towel. Roots were then cut into 2.5-cm (1.0 in.) pieces, and 2.0 g were tightly packed inside a pre-weighed, heat-resistant cylindrical plastic jar measuring 3.0-cm height × 2.9-cm diameter (1.2 × 1.1 in.) (Fig. 5.1). Each plastic jar had four equally spaced 3.2-mm (0.13 in.) diameter entry holes drilled in its walls at 90° apart, located 1.0- cm (0.4 in.) above its base. Each hole was spaced 2.3 cm (0.91 in.) from the adjacent hole, allowing termites to enter the jar from the surrounding substrate from several directions (Fig. 5.1A). An additional entry hole was drilled in the bottom center of each jar (Fig. 5.1B). Plant material was placed into each open jar, air-dried for five days, and the jar with plant material was weighed. In addition, each plant-packed jar was oven-dried for 24.0 hours at 80°C (176°F) and a dry weight obtained. These jars were then placed in a large open container under ambient laboratory conditions for 10.0 days to re-absorb moisture. A near-homogenous mixture of stems and leaves were prepared in the same manner.

**Choice Study.** In the choice bioassay of roots, or stem-and-leaf mixtures, 18 cylindrical plastic containers, each measuring 15.2-cm diameter × 6.4-cm height (6.0 ×
2.5 in.) with a removable lid were used to complete six replicates for each of three termite
groups (Kard et al. 2007) (Fig. 5.2). To allow for air exchange, two 3.2-mm-diameter
holes (0.13 in.) were drilled into each container lid at 6-cm (2.4 in.) from the lid edge,
and spaced 3.5 cm (1.4 in.) apart (Fig. 5.2A).

**Substrate.** Each container received 307 g of substrate consisting of sterilized white
sand mixed with vermiculite mixed in a 10:1 ratio (w/w). The white sand and vermiculite
mixture was oven-dried at 103°C (217.4°F) for 24 hours. Sterile water was added at a
rate of 350 ml per 1,000 g of dry substrate, resulting in a moisture content of 26% (w/w).
The substrate was tamped down to provide a level surface (Kard et al. 2007).

Four jars, each containing roots of one of the four plant species alone, and four additional
jars each containing stem-and-leaf mixture of one of the four plant species, were
randomly placed on top of the substrate and arranged in a circle within each container.
Each jar was equidistant from the adjacent jars, and 1.0 cm (0.39 in.) from the interior
wall of the container (Fig. 5.2B). Each jar was pressed down into the substrate so that all
but the upper 0.5 cm (0.195 in.) was buried. Each container received 1,000 worker and 30
soldier termites (when enough soldiers were available) (Kard et al. 2007).

**No-Choice Study.** No-choice bioassays were conducted for the individual roots alone
and for the stem-and-leaf mixtures of the four plant species, totaling eight different food
choices. Additionally, a 2.0-g oven-dried block of *P. radiata* measuring 2.0 × 2.0 × 1.5
cm (0.8 × 0.8 × 0.6 in.), processed in the same manner as the plant material was used as
the control. The bioassay for each test material consisted of 18 plastic cylindrical
containers, each measuring 10.3-cm diameter × 8.9-cm height (4.1 × 3.5 in.) with a
removable lid, resulting in six replicates per termite group per material (Kard et al. 2007)
(Fig. 5.3). To allow for air exchange, two 3.2-mm (0.13 in.) diameter holes were drilled in each container lid, 1.0 cm (0.4 in) from the circular peg handle and 2.5 cm (1.0 in.) from the lid edge, and spaced 5.5 cm (2.2 in.) apart (Fig. 5.3A). Each container held 110 g of substrate tamped down to a level surface (Kard et al. 2007). One jar with a designated bioassay food choice was placed 1.0 cm (0.39 in.) from the interior wall of the container and pressed into the substrate so that all but the upper 0.5 cm (0.20 in.) was buried. Each container then received 250 worker and 10 soldier termites when enough soldiers were available (Fig. 5.3B).

After 12 weeks the surviving termites in each container were counted. Remaining plant material was cleaned of plastering and debris, placed in a glass petri dish, oven-dried for 24 hours at 80°C (176°F), and a dry weight obtained.

**Termite Identification**

**Morphological Techniques.** Termite samples were collected and preserved in vials containing 70% or 100% ETOH. Soldiers and alates, when available, were identified morphologically using standard keys (Banks 1946; Gleason and Koehler 1980; Scheffrahn and Su 1994; Brown et al. 2005). Measurements of body components were obtained using an Olympus SZ61 stereo microscope utilizing SPOT software version 4.6.4 (Diagnostic Instruments, Center Valley, PA).

**Molecular Techniques.** Termites were also molecularly identified. Polymerase chain reaction (PCR) amplification of a 428-bp region of the mtDNA 16S rRNA gene was achieved using universal primers: forward primer LR-J-13017 (5’-TTACGCTGTTATCCCTAA-3’) (Austin et al. 2005); reverse primer LR-N-13398 (5’-CGCCTGGTTATCAAAAAACAT-3’) (Austin et al. 2005). One-to-two termites,
depending on availability, were removed from each sample and placed on filter paper to dry. Dried termite(s) were then placed into a 1.5-ml micro-centrifuge conical tube, and pulverized using a Pellet Pestle (VWR Scientific, West Chester, PA). Termite DNA was then extracted using DNeasy® Blood and Tissue kit 69504 (Qiagen, Germantown, MD). Termite samples were allowed to incubate in proteinase for thirty minutes at 56°C. The extracted DNA was eluted with 50µl of Buffer AE instead of the suggested 100µl, to increase the final concentration of DNA. A master mix consisting of FastStart PCR Master (Roche, Indianapolis, IN) primers and water were prepared in a 1.5µl micro-centrifuge conical tube. A 24µl aliquot of master mix was placed into PCR microtubes, to which 1µl of extraction template (DNA) from each sample was added. FastStart contains the reagents Taq DNA polymerase, magnesium chloride, double-concentrated reaction buffer, and nucleotides (dNTPs). The PCR profile consisted of 1 cycle at 95°C for 4 min., 46°C for 45 s, 72°C for 45 s, followed by 34 cycles at 95°C for 45 s, 46°C for 45 s, and 72°C for 45 s. Amplification of DNA was performed using an MJ Mini Thermal Cycler (BIO-RAD, Richmond, CA). The quantity of PCR product present and 260/280 ratio for each sample was determined using the NanoDrop Spectrophotometer located in the Oklahoma State University Biochemistry Microarray Core Facility. Any PCR product sample with < 10 ng/µl and/or a 260/280 ratio of < 1.70 was re-extracted. PCR product was cleaned of excess primers and dNTPs using ExoSap-IT® (USB Corporation, Cleveland, OH).

A 2% agarose gel was prepared using 100 ml of TBE buffer and four 500 mg agarose tablets (Amresco, Solon, OH), then 5µl of ethidium bromide was added to each gel. Each gel was loaded with 2µl of low molecular ladder and 2µl of cleaned PCR product, and
then mixed with 1.0µl of Bromophenol blue gel dye. The loaded gel was run on a
FisherBiotech Gel Electrophoresis system (Fisher Scientific, Pittsburg, PA). Each gel
was viewed using a MultiDoc-It Digital Imaging System (UVP, Upland, CA) to insure
the presence of DNA and to quantify the amount of DNA present. Samples (10 µl) of
PCR product at a concentration of 5 pmole/µl were transported to the Oklahoma State
University Recombinant DNA/Protein Resource Facility for sequencing. Samples were
sequenced using the Applied Biosystems BigDye® Terminator version 1.1 Cycle
Sequencing Kit, and analyzed with Applied Biosystems Model 3730 capillary DNA
sequencer (Applied Biosystems, Foster City, CA). DNA sequences for R. flavipes, R.
tibialis, R. hageni, R. virginicus voucher specimens were submitted to BLASTn (Basic
Local Alignment and Search Tool, nucleotide) and queried against their respective
species in the database of 16S sequences (Szalanski et al. 2004). Resulting sequences
were used to establish consensus sequences using ClustalW program at EMBL-EBI
(European Bioinformatics Institute, UK). Sequences from all remaining samples were
submitted to NCBI (National Center for Biotechnology Information), and a BLASTn
search using Genbank accession numbers was used to validate species.

Statistics. Analyses were performed with SAS Version 9.4 (SAS Institute, Cary,
NC). Analysis of variance (PROC MIXED) was used to assess the effect of source and
colony. A two-factor factorial arrangement in a completely randomized design was
utilized. Factor level means and standard errors are reported, and simple effects are
compared with protected pairwise comparisons.
Results and Discussion

Choice Study. Feeding preferences of the four most predominant TGPP grasses and their roots in the choice study were significantly different (Table 1) (Fig. 5.4). Termites tunneled through the substrate and plastered feeding jars within the first week of the study. Lids to the jars were removed after the first two weeks to better observe food consumption and activity (Fig. 5.5). Termites plastered over the food and top of the jar with tunnels leading to the substrate. Consumption weights of four grasses or roots, listed in descending order of the first to least preferred based on weight consumed are: SWR, BBR, BBG, ING, INR, LBR, SWG and LBG (Fig. 5.4). *R. tibialis* preferred SWR (Fig 5.6) and least preferred LBG (Fig. 5.4). Although roots were consumed in greater amounts compared with stem and leaf mixtures, this does not mean that grass options were not palatable. We observed termites hollowing out the stems of BBG and ING (Fig. 5.7A, B, C). As stated above, both BBG and ING were the most preferred stem-and-leaf mixtures. It is possible that termites in the TGPP are tunneling and feeding within the tall stems of both species of grasses, which would give them protection from predators and avoid desiccation. It is interesting to note that in the biomass study, above-ground plant biomass increased in field plots containing termites. However, neither plots with nor without termites showed differences in soil chemistry: pH, buffer index, surface NO$_3$-N, phosphorus, potassium, organic material, total carbon and total nitrogen were similar (Smith, M.P., pers com). There was also no difference in the ratio of carbon-to-nitrogen ratio. There were no differences in soil bulk density, soil moisture content, or water infiltration rates. This would imply that the difference in plant biomass between baited and non-baited plots is not completely due to the above factors, but that other soil
chemistry and physical factors are involved in differences in plant biomass. Future research on the presence of termites within grass stems on the TGPP is needed. To provide the termites a choice of only the foods normally accessible on the TGPP, pine blocks used as controls in the no-choice test were not included in the choice test.

**No-Choice Study.** Feeding preferences of the four predominant TPGG grasses and their roots, and the pine block were significant (Table 2) (Fig. 5.8). Similar to choice tests, termites in the no-choice study tunneled through the substrate and plastered feeding jars within the first week. Jar lids in the no-choice study were also removed after the first two weeks in order to better observe termite food consumption and activity (Fig. 5.5). Termites plastered over the food and top of the jar with tunnels leading to the substrate. Consumption weights, showed in order of the most to least preferred are: INR, BBR, BBG, LBR, P, SWG, ING, SWR, LBG (Fig. 5.8).
Fig. 5.1. Plastic feeding jar (2.9-cm diameter $\times$ 3.0-cm height): (A) four 3.2-mm diameter entry holes on side wall 90° apart; (B) one hole through the bottom.
Fig. 5.2. Top view of 15.2-cm diameter × 6.4-cm tall choice feeding container: (A) two 3.2-mm diameter air exchange holes through lid; (B) eight 2.9-cm diameter × 3.0-cm height interior plastic jars containing roots, or stems and leaves of one of the four grass species.
Fig. 5.3. No-choice feeding container (10.3-cm diameter × 8.9-cm height): (A) two 3.2-cm diameter air vent drill holes in lid; (B) interior jar (2.9-cm diameter × 3.0-cm height) with roots, or stems and leaves of one of four grass species.
Table 5.1. Mean ± SE weight difference (g) for a 12 week choice feeding study

<table>
<thead>
<tr>
<th>Food Source</th>
<th>Consumption Comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBG</td>
<td>0.78 ± 0.063bc</td>
</tr>
<tr>
<td>BBR</td>
<td>0.84 ± 0.070b</td>
</tr>
<tr>
<td>ING</td>
<td>0.65 ± 0.026cd</td>
</tr>
<tr>
<td>INR</td>
<td>0.64 ± 0.041cd</td>
</tr>
<tr>
<td>LBG</td>
<td>0.36 ± 0.035e</td>
</tr>
<tr>
<td>LBR</td>
<td>0.52 ± 0.075de</td>
</tr>
<tr>
<td>SWG</td>
<td>0.50 ± 0.021de</td>
</tr>
<tr>
<td>SWR</td>
<td>1.10 ± 0.107a</td>
</tr>
</tbody>
</table>

Two means with the same letter are not significantly different at a 0.05 level of significance.
**Fig. 5.4.** Choice feeding study consumption comparison of four different grass and four different root species by 1000 termites for 12 weeks. Means with the same letter are not significantly different ($\alpha=0.05$).

**Key:** BBG (Big Bluestem leaves and stems), BBR (Big Bluestem root), ING (Indiangrass leaves and stems), INR (Indiangrass root), LBG (Little Bluestem leaves and stems), LBR (Little Bluestem root), SWG (Switchgrass leaves and stems), SWR (Switchgrass root)
Fig. 5.5. Termites feeding on Indiangrass root.
Fig. 5.6. Termites feeding on Switchgrass root.
Fig. 5.7. (A) Termites feeding on big bluestem stem; (B) Termite damage to big bluestem stem; (C) Termites feeding on big bluestem leaves.
Table 5.2. Mean ± SE weight difference (g) for a 12 week no-choice feeding study

<table>
<thead>
<tr>
<th>Food Source</th>
<th>Consumption Comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBG</td>
<td>0.67 ± 0.027b</td>
</tr>
<tr>
<td>BBR</td>
<td>0.85 ± 0.077a</td>
</tr>
<tr>
<td>ING</td>
<td>0.52 ± 0.027bc</td>
</tr>
<tr>
<td>INR</td>
<td>0.97 ± 0.069a</td>
</tr>
<tr>
<td>LBG</td>
<td>0.48 ± 0.026c</td>
</tr>
<tr>
<td>LBR</td>
<td>0.66 ± 0.114b</td>
</tr>
<tr>
<td>SWG</td>
<td>0.54 ± 0.027bc</td>
</tr>
<tr>
<td>SWR</td>
<td>0.49 ± 0.017c</td>
</tr>
<tr>
<td>P</td>
<td>0.66 ± 0.038b</td>
</tr>
</tbody>
</table>

Two means with the same letter are not significantly different at a 0.05 level of significance.
Fig. 5.8. No-choice feeding study consumption comparison of four different grass and four different root species by 1000 termites for 12 weeks. Means with the same letter are not significantly different ($\alpha=0.05$).

**Key:** BBG (Big Bluestem leaves and stems), BBR (Big Bluestem root), ING (Indiangrass leaves and stems), INR (Indiangrass root), LBG (Little Bluestem leaves and stems), LBR (Little Bluestem root), SWG (Switchgrass leaves and stems), SWR (Switchgrass root)
Acknowledgements

Thanks to Dr. Hassan Melouk for the use of the greenhouse. Zachary and Brittani Smith for their assistance in watering plants.
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CHAPTER VI

SUMMARY AND CONCLUSIONS
Results from the research projects described in chapters three, four, and five answered some questions that TGPP termite researchers have pondered since subterranean termite studies started there in 2002. In chapter three, we examined the effects of *Reticulitermes* sp. on root biomass and above-ground plant growth in sites with active termite foraging populations (non-baited study plots) and compared with sites where termites were eliminated (toxicant-baited study plots). Results showed that plots containing termites supported greater above-ground plant growth compared with plots where termites were absent. A noteworthy discovery is that the root biomass in both non-baited and baited plots was similar, exhibiting no significant differences for the study duration. Most likely, this is because termites are primarily feeding on the roots in a steady-state ecological balance, thereby stimulating overall plant growth. Therefore, a measurable increase in root growth may be negated and balanced by removal of root tissue by foraging termites, resulting in no increase in root biomass. Research conducted in other countries reported increased plant growth in areas with termites, due to termites adding nutrients and aiding in water infiltration (King 1977, Woodell and King 1991, Dean et al. 1997, Blomqvist et al. 2000, Holt and Lepage 2000, Folgarait et al. 2002, Jouquet et al. 2004, Flores-Palacios and Ortiz-Pulido 2005, Jouquet et al. 2005, Jouquet et al. 2006, Garba et al. 2011). However, concurrent research on the TGPP reports no difference in soil nutrients or water infiltration rates when comparing non-baited with baited plots (M. Smith, pers. com), implying feeding on roots by the termites is stimulating growth.

In chapter four, results did not indicate any foraging by termites for above-ground litter on the TGPP. No termites or evidence of termite activity were observed around the mesh bags containing plant material, in either baited or non-baited plots, above or below-
ground. This lack of termite foraging may have been due to the extreme drought conditions during the time of this research project, as the termites may have remained 15 cm or more below the soil-surface in more hospitable conditions. The explanation of why in the past termites were observed on the soil-surface, but not during the mesh bag study, needs to be investigated by future researchers during more normal weather conditions. There was no significant difference between mesh bag plant content in non-baited compared with baited plots. However, there was a significant difference in plant content in above-ground mesh bags compared with below-ground mesh bags in both non-baited and baited plots. Below-ground bags retained more moisture during the brief periods of rain, increasing fungal growth and subsequently resulting in the loss of plant material within the mesh bag.

In chapter five results of the choice feeding study indicated that termites preferred switchgrass roots (SWR). Indiangrass root (INR) was preferred in the no-choice study. In both choice and no-choice studies, little bluestem stems and leaves (LBG) were least preferred. Results showed termites preferred to feed on roots over stems and leaves. This may be due to roots containing more moisture and possibly more nutrients than leaves and stems. One of the most exciting finds of this study was the laboratory study observation of termites feeding inside big bluestem stems (BBG). This feeding behavior demonstrates that termites will forage for food and moisture within the protective internal confines of the stems. It would be interesting for future researchers to evaluate if termites can be found foraging within these stems in the field. These studies provide valuable new information regarding food sources of subterranean termites on the TGPP.
References Cited


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Doctor of Philosophy

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