OKLAHOMA FORMOSAN SUBTERRANEAN

TERMITE SURVEILLANCE PROGRAM

AND TERMITE

SURVEY

By

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CHAPTER I

INTRODUCTION

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is an exotic structural pest that is steadily spreading in the United States, causing extensive damage to wooden structures and wood products. One billion dollars is spent annually on damage caused by the Formosan subterranean termite. The Formosan subterranean termite costs Louisiana home and building owners more than $350 million annually in damage, repairs and prevention (USDA ARS 2006). In the Hawaiian Islands, the Formosan subterranean termite is the number one structural pest, damaging homes, boats, utility poles, and underground electrical and telephone cables (Tamashiro et al. 1987). Since its first noted introduction into the southeastern continental United States in 1957 (Chambers et al. 1988, Cabrera et al. 1991), the Formosan subterranean termite has spread as far north as Denton County, Texas (Howell et al. 2001), 21 miles south of the Red River along the Oklahoma-Texas border. The Formosan subterranean termite has also been found east of Denton County in adjacent Collin County (Center for Urban & Structural Entomology 2007). The potential negative economic impact of this termite to Oklahoma home and business owners, as well as the forest products industry, compelled initiation of a statewide early detection surveillance program in 2005. During 2005 through 2007, detection devices consisting of soil-surface ground-boards, in-ground monitoring devices, and elevated light traps were installed throughout the southern Oklahoma counties of Jackson, Tillman, Cotton, Jefferson, Love, Marshall, Bryan, Choctaw and McCurtain. Additionally, county extension agents and pest management professionals cooperated in the detection effort by collecting termites from structures. Inspections of landscaping timbers and railroad crossties were also regularly conducted at commercial outlets in Oklahoma counties of Woodward, Garfield, Custer, Beckham, Comanche, Carter, Oklahoma, Pittsburg, Payne, Creek, Osage, Washington, Tulsa, Rogers and Craig. In southern states these imported products often contain Formosan subterranean termites (LSU AgCenter Research & Extension 2004).

The Formosan subterranean termite is endemic to the island of Formosa (Taiwan), as well as China and Japan (Cabrera et al. 1991). Since first reported in the continental United States in Charleston, South Carolina in 1957, it has become established in at least eleven states (Chambers et al. 1988, Cabrera et al. 1991). This termite is an aggressive wood destroyer, with colonies feeding on a wide variety of hosts, including live plants. The ability of this termite to attack and kill living plants and trees is a primary reason that on-going surveys and continuous vigilance are required. Early detection and intervention may prevent its establishment in Oklahoma. Due to its diverse food choices and ability to survive above-ground for long periods, colonies can be transported to other geographic regions in landscaping timbers, crates, potted plants, pallets, wooden boats, motor homes, and all manner of human commerce (Cabrera et al. 1991). Hundreds of millions of dollars are spent each year in the United States for structural repairs and prevention.

The distribution of endemic termite species throughout Oklahoma is not well known. A survey by Brown et al. (2004) documented termite species in 23 Oklahoma Counties, from personal collections and earlier reports. The Formosan subterranean termite survey allowed for a concurrent survey for endemic species.

Termites collected during the surveillance program that is the subject of this thesis were identified and their distribution added to the Oklahoma termite distribution map by Brown et al. (2004). Morphological identification of termites utilizes dichotomous keys for soldiers and alates (Banks 1946, Snyder 1954, Nutting 1990, Scheffrahn and Su 1994). Overlapping morphological characteristics of *Reticulitermes flavipes* (Kollar) and *R. virginicus* (Banks) soldiers makes positive identification difficult. Samples that contain workers only make morphological identification impossible as no keys for workers exist. Advancements in molecular techniques using genetics make positive identification possible, regardless of termite caste.

The overall goal of this research was to monitor Oklahoma for presence of the Formosan subterranean termite, expand the known distribution of endemic termite species, and use morphological and molecular techniques to positively identify termites to species.

Objectives

I. Initiate and conduct a surveillance program for the Formosan subterranean termite.

II. Expand the Oklahoma termite survey.

III. Identify and validate Oklahoma termite species morphologically and with molecular techniques, using a region of the mtDNA 16S rRNA gene.

CHAPTER II

REVIEW OF LITERATURE

General Termite Introduction

Termites are in the order Isoptera. They are eusocial insects that have three castes: primary reproductives (founding queen and king), workers, and soldiers. Caste members may be either male or female.

Workers are the most numerous caste members and are responsible for foraging for food, nursery maintenance, larval care, building mud tunnels, and grooming nestmates (Lee and Wood 1971, Thorne and Forschler 1999, Potter 2004). In some species, workers also help defend the colony by attacking and biting 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). A colony numbering in the millions can consume as much as 0.5 kg (1.1 lb.) of wood per day (Grace 1992). Workers may live up to two years depending on environmental conditions (Potter 2004).

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

Alates are winged reproductives (swarmers) that disperse from their parent colony to establish new colonies. For different termite species, flight distances may 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 (3,000 ft) (Light 1934). After these nuptial dispersal flights, both female and male alates will break off their wings at a suture line present in the wing. 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 a 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 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. Abdominal expansion increases over time as the size of the ovaries increase, and fecundity increases due to the urgency of the 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 possess sclerotized heads (Harris 1961). Both nymphs and workers can develop into supplementary reproductives (neotenics) that stay within the colony. Supplementary 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 fly away 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).

History and Distribution of the Formosan Subterranean Termite

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) is one of 28 known species in the genus *Coptotermes*, and worldwide it is the most economically important termite pest (Su and Scheffrahn 2005). A colony of Formosan subterranean termites can contain over a million termites with a foraging area the length of a football field (Su and Scheffrahn 1988a). Geographically, it ranges from 35° north to 35° south of the equator, and prefers temperatures between 17°C and 32°C with a relative humidity between 56% and 72% (Su and Tamashiro 1987, Gold et al. 2005). It originated from the island of Formosa, now known as Taiwan, and from mainland China and Japan. As of 2007, the Formosan subterranean termite has been reported in China, Guam, Japan, Midway Island in the North Pacific, South Africa, Sri Lanka, Taiwan, and the United States including the Hawaiian islands (Su and Tamashiro 1987, Hu 2003). The Formosan subterranean termite is believed to have been transported to the Hawaiian Islands around 1907 during the sandalwood trade with China (Su and

Tamashiro 1987). It was first reported in the United States in 1965 in Houston, Texas. However, the first Formosan subterranean termites found in the continental United States were collected in Charleston, South Carolina, in 1957. This termite was first misidentified as *Incisitermes schwartzi* (Banks) at that time. However, in 1969, these termites were correctly identified as *C. formosanus* (Chambers et al. 1988). In the United States, Formosan subterranean termite infestations have been found in Alabama, Florida, Georgia, Hawaii, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, Texas, and San Diego, California (Potter 2004).

Mode of Introduction and Spread of the Formosan Subterranean Termite

The Formosan subterranean termite can be introduced to new locations in wooden containers, crates, pallets, wooden boats, floating logs (Su and Tamashiro 1987), and railroad crossties (USDA ARS 2006). Long distance transport of the termite can occur through the worldwide movement of boats, especially where adequate moisture is contained within the inner hull (Coaton and Sheasby 1976). Alate wings are covered with minute hairs that are believed to help these reproductives disperse along the water by allowing them to float on the water surface where they can drift to the riverbank or climb onto floating logs (LSU AgCenter Research & Extension 2005a). From 1993 through 1997, five out of seven reports involving the Formosan subterranean termite in the Atlanta, Georgia, metro area were related to infested railroad ties (Forschler et al. 2001). Formosan subterranean termites can also be transported in the soil of potted shrubs and trees purchased from nurseries and home and garden centers. Over 50 species of plants in Hawaii (Tamashiro et al. 1987), and over 30 different plant species in the continental United States, have been attacked by the Formosan subterranean termite (LSU AgCenter Research & Extension 2005b). In 1992, San Diego County, CA, reported *Coptotermes formosanus* in a residential home located in La Mesa. The landscaped area of this home was well irrigated and included live fruit trees, creating an ideal habitat for the Formosan subterranean termite to become established. Evidence of Formosan alates within this location indicated a mature colony. This introduction is believed to have occurred in June 1976, when neighbors moved into the area from Hawaii and brought potted plants and other outdoor wooden containers with them. The Formosan subterranean termite also spreads naturally by swarming. Swarming alates were responsible for infesting four other homes within the immediate area (Atkinson et al. 1993).

Biology and Ecology of the Formosan Subterranean Termite

Swarming. Generally, a Formosan subterranean termite colony can produce over 70,000 alates that will swarm sometime during May through August (differs per location) in warm, humid conditions at dusk, and they are positively phototrophic (Su and Scheffrahn 2005). Several environmental factors determine if a swarm will occur: temperature, light intensity and wind velocity. Ideal swarming temperatures are 20-to-30°C (Su and Tamashiro 1987, Tamashiro et al. 1987). A light intensity of 10.8 lumen/m^2 is ideal. However, if light intensity drops to 0.14 lumen/m^2 or less, swarming will cease. Wind velocity is the main environmental factor influencing flight initiation. Wind velocities below 3.7 km/h are ideal, whereas at wind velocities above 3.7 km/h flights will cease (Leong et al. 1983). Alates have been recorded flying as far as 460 m at a speed of 2.2 m/sec. Wind currents allow them to reach heights greater than 40 m, imparting the potential to infest upper floors of high-rise buildings (Messenger and Mullins 2005). However, some alates may only fly a few meters before dropping to the ground, where they break off their wings. Male (king) and female (queen) will pair-up and generally seek a moist, warm crevice in or adjacent to wood prior to mating. A mature colony may develop in three-to-five years, producing 1-to-10 million termites (Hu 2003).

Foraging and Nutrients. The Formosan subterranean termite is known mostly as a serious structural pest that feeds upon materials containing cellulose, i.e., fabric, paper, cardboard, books, fence posts, utility poles, plants, and human dwellings (Lai et al. 1983, Mauldin 1986). Foraging distances can extend over 100 m (328 ft.) (Potter 2004). Formosan subterranean termites are relatively aggressive and can overrun a *Reticulitermes* sp. colony (Su and Scheffrahn 1988b). Due to the relatively large numbers of the colony, they cause more structural damage in less time compared with *Reticulitermes* sp. (Su and Scheffrahn 2005). Termites require moisture to survive and obtain it from condensation inside structures, or from roof or plumbing leaks (Hu 2003).

The Formosan subterranean termite will consume a wide variety of living plants, and many plants offer different levels of nutritional value that may influence colony survival. For *Reticulitermes* sp., the nutritional value of living plants is not as critical (LSU AgCenter Research & Extension 2005a). In a laboratory test, Formosan subterranean termites that were fed pecan [*Carya illinoensis* (Wangenh.)] or red gum (*Liquidambar styraciflua* L.) wood produced more progeny than those fed other wood species. Termites fed ponderosa pine (*Pinus ponderosa* Laws.) or loblolly pine (*Pinus taeda* L.)

had a lower survival rate of progeny and produced fewer workers and soldiers than those fed other wood species. This type of information may prove valuable for developing future control measures (Morales-Ramos and Rojas 2003). The Formosan subterranean termite feeds on over 30 species of living plants in the continental United States (LSU AgCenter Research & Extension 2005b). In Louis Armstrong Park, New Orleans, LA, 32% of the trees (24 tree species) were infested by Formosan subterranean termites (Messenger and Su 2005). In Charleston, South Carolina, the presence of Formosan subterranean termites was noted in 17 species of trees. Of these 17 tree species, 14 are also found in Oklahoma. These trees could be threatened by Formosan subterranean termites if they invade the state. Susceptible trees include elm (*Ulmus sp*.), hackberry (*Celtis occidentalis* L.), black cherry (*Prunus serotina* Ehrh.), sycamore (*Platanus occidentalis* L.), southern magnolia (*Magnolia grandiflora* L.), crape myrtle (*Lagerstroemia indica* L.), oak (*Quercus sp*.), american beech (*Fagus grandifolia* Ehrh.), redbud (*Cercis sp*.), and flowering dogwood (*Cornus florida* L.) (Harlow and Harrar 1968, Chambers et al. 1988, Seiler and Peterson 2008). Formosan subterranean termites are also found in water-bound trees such as the Baldcypress [*Taxodium districhum* (L.) Rich.], which occurs in McCurtain and Le Fore counties in Oklahoma (Harlow and Harrar 1968, Seiler and Peterson 2008). *Reticulitermes* sp. also feed in live trees, but do not forage through the xylem or cause structural weakness like the Formosan subterranean termite (LSU AgCenter Research & Extension 2005a).

Nesting. Formosan subterranean termite workers build a hard, sponge-like dark-brown above-ground nest termed 'carton', that is made of soil and masticated plant material cemented together with saliva and excrement (La Fage and Nutting 1978, Chen et al. 1998). Carton nests provide a moist environment, thus these termites can sustain themselves above-ground for extended periods without having to return to the soil for moisture. Moisture within a carton nest can be obtained from leaky roofs, or plumbing and air conditioning condensation (Su and Scheffrahn 1990). Colonies containing millions of termites may construct more than one carton nest. The main carton nest usually contains the primary king and queen. Satellite carton nests usually contain workers and soldiers that are foraging away from the main carton nest. Satellite carton nests are known to contain as many as 75 neotenic queens (Coaton and Sheasby 1976). Carton nests can be found in attics, wall voids, high-rise apartments, trees, and chimneys, to name a few locations (Hu 2003). In southeastern Florida, aerial nests account for 25% of structural infestations. In Hawaii, aerial nests account for 50% of infestations (Su and Scheffrahn 1986, Tamashiro et al. 1987). *Reticulitermes* sp. do not build a carton nest, but will extend their mud shelter tubes in search of food and water (Su et al. 2007).

Formosan Subterranean Termite Identification

Formosan subterranean termite alates are 12-to-15 mm in length including their wings, have yellowish-brown body, and clear wings covered with minute hairs. Two thick parallel veins extend longitudinally along the wing costal margin (Jones and Howell 2000, Gold et al. 2005). Workers are creamy-white with a light-orange-colored head, and feed and groom the soldiers and king and queen as well as other workers. They also maintain the nest and care for the brood. Soldiers have a creamy-white body, orange-brown-colored oval-shaped head, and crossed sickle-shaped mandibles without teeth that are used for defense against predators (Cabrera et al. 1991, Chen et al. 1999, Jones and

Howell 2000, Su and Scheffrahn 2005). The soldier head capsule has a pronounced fontanelle, a frontal gland pore, dorsal and centered at the base of the mandibles. When threatened, soldiers can excrete a glue-like substance from the fontanelle, hindering the mobility of the enemy. Often the soldier is also entangled in this excretion and dies (Abe et al. 2000). Excretion components include lignoceric and hexacosanoic free fatty acids (Ohta et al. 2007). The fontanelle excretion also acts as an alarm pheromone, alerting other soldiers to congregate at the site of enemy attack (Eisner et al. 1976). According to Ohta et al. (2007) the excretion indicates a loss of soldiers and may stimulate workers to become soldiers.

Collecting Formosan Subterranean Termites

Unlike *Reticulitermes* sp. endemic to Oklahoma, Formosan subterranean termite alate flight occurs at dusk from April through July (varies with location). Because Formosan subterranean termites are attracted to light they are often found around window sills and porch lights. Collecting alates can be a challenge in areas where light sources are unavailable, such as wooded areas or areas without electrical service. Solar-powered landscaping lights, such as Brinkmann Solar Renaissance III (Dallas, TX), and white sticky boards were used to capture alates in southern Mississippi from 2001 to 2003 (Etheridge et al., unpublished data). These light traps used light-emitting diode (LED) lights and trapped 7,489 Formosan subterranean termites in 2001 (110 solar light traps), 4,593 in 2002 (144 solar light traps) and 9,937 in 2003 (123 solar light traps). This trap configuration was not only an effective tool for capturing termite alates, it was less expensive than conventional light traps (Etheridge et al., unpublished data).

Formosan subterranean termites (as well as *Reticulitermes* sp.) can be collected using in-ground and soil-surface traps. Wood placed on the soil surface and covered with a large container or bucket can attract thousands of termites (Tamashiro et al. 1973). Another trap consists of 10.3-cm (4.0 in.)-diameter polyvinyl chloride (PVC) pipe that is cut into 20.3-cm (8.0 in.) sections. Each pipe section has four equally spaced, parallel longitudinal rows of 12 drilled 3.2-mm (0.125 in.)-diameter holes (Brown et al. 2004). Each hole is spaced 1.3 cm (0.50 in.) from adjacent holes and allows termites to enter the PVC pipe from the surrounding soil. Each pipe section is inserted vertically into a 15.2-cm (6 in.) pre-drilled hole in the ground (Brown et al. 2004). A wood matrix sandwich, consisting of seven parallel pine slats, each measuring 17.8 x 6.4 x 0.6 cm (7.0 x 2.5 x 0.25 in.), and separated by tongue depressors, are bound together with galvanized steel wire. The tongue depressors provide space for termite movement between slats. Each sandwich is wrapped with a piece of cardboard, 37.5 cm x 18.5 cm (14.8 x 7.3 in.) and then inserted into the pipe. A 10.2-cm (4.0 in.)-diameter PVC cap is placed on top of each pipe to protect the contents from predators and rain (Brown et al. 2004). These types of traps provide an excellent way to visualize colony activity.

Termites Endemic to Oklahoma

There are five families of termites in the United States: Hodotermitidae, Kalotermitidae, Rhinotermitidae, Termitidae, and Termopsidae (Weesner 1965, Thorne et al. 1993). Rhinotermitidae and Termitidae (subterranean termites) are 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).

Reticulitermes flavipes is considered the most important economic termite pest in the United States (Potter 2004). *R. flavipes* colonies may contain 100,000 to 3,000,000 individuals, and forage up to 71 m (232 ft) from the main nest (Grace et al. 1989, Su et al. 1993). Alate bodies of *R. flavipes*, *R. virginicus,* and *R. tibialis* are dark brown to black (Su et al. 2007). *R. hageni* has a yellowish-brown body and overall length ranging from 7-8 mm (including wings), and is occasionally misidentified as *C. formosanus* (12-15 mm including wings) due to their similar body color. *R. flavipes* (10 mm including wings) on average is larger than *R. virginicus*, 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. *Reticulitermes* wings are similar to the Formosan subterranean termite, but lack the minute surface hairs (Gleason and Koehler 1980).

G. tubiformans is a desert termite found on prairies or in semi-arid regions of Arizona, Northern Mexico, and Texas (Allen et al. 1980). Although considered a pest by ranchers due to the amount of vegetation they consume, they are valuable decomposers of organic matter (Mackay et al. 1987). *G. tubiformans* lives in the soil in pastures and grasslands, and is sometimes found under rocks and cow manure. Food includes cow manure and vegetation, including but not limited to grass roots and stems, Honey mesquite, Blue grama, and Buffalo grass. This termite covers its food with mud sheets or tubes, constructed at night and after significant rainfall, that 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 long and straight, but are turned inward at the 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), and swarm in spring and summer after rain storms (Snyder 1954, Gleason and Koehler 1980, Nutting 1990).

Molecular Techniques

Whereas identification of termites to species can be difficult due to overlapping morphological measurements between 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). The 16S gene was also used to determine genetic variation and to design PCR primers specific to *C. formosanus* (Szalanski et al. 2004). *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, and 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.

CHAPTER III

OKLAHOMA FORMOSAN SUBTERRANEAN TERMITE SURVEILLANCE PROGRAM AND TERMITE SURVEY

Materials and Methods

Monitoring Devices. Cylindrical in-ground monitoring devices (Figs. 3.1a, b) are made of polyvinyl chloride (PVC) pipe and measure 20.3-cm (8.0 in.)-tall by 10.2-cm (4.0 in.) inside diameter. Each pipe has four longitudinal equally-spaced parallel vertical rows consisting of twelve drill holes. Drill holes measure 3.2-mm (0.125 in.) in diameter and are longitudinally spaced 1.3-cm (0.50 in.) apart. A wood sandwich consisting of seven 17.8 x 6.4 x 0.6-cm (7.0 x 2.5 x 0.25 in.) *Pinus* sp. slats, each oriented lengthwise and parallel with each other, and separated by a tongue depressor, are bound together using three 27.9-cm (11 in.) plastic cable ties. The slat sandwich is wrapped with a rectangular piece of corrugated cardboard measuring 37.5 x 18.5 cm (14.8 x 7.3 in.). Each PVC pipe was inserted vertically into a 17.8-cm (7.0 in.)-deep by 10.2-cm (4.0 in.)-diameter hole, pre-drilled into the soil with a power auger. Once inserted into the hole, soil was packed around the exterior perimeter of the pipe to ensure soil-to-pipe contact and a stable installation. A PVC cap is placed on top of the pipe (Fig. 3.1c) (Brown et al. 2004).

Rectangular soil-surface ground-boards are made of pine, measuring 30.5 x 15.2 x 2.5 cm (12.0 x 6.0 x 1.0 in.). Vegetation is removed and each board is placed directly on the bare mineral soil surface. A standard building brick is placed on to each board to hold it in place and ensure solid contact with the soil surface (Fig. 3.1d) (Brown et al. 2004).

In remote areas where there were no permanent man-made light sources, elevated solar-powered LED light traps were used to potentially attract Formosan subterranean termite alates, if present. A unique light trap was developed and constructed for use in this study and named "Smith Light Trap" (Figs. 3.2a, b). This light trap consists of a translucent Lucite-Tuf® (Lucite International, Inc., Cordova, TN) high-impact rectangular acrylic top measuring 56.0 x 20.0 x 0.2 cm (22.0 x 8.0 x 0.08 in.). This top excludes rain and allows passage of light to energize the two solar-powered lights contained within the trap. This removable top is affixed with screws to a rectangular box wooden frame, providing ready access into the trap. A 25.4 x 10.2-cm (10.0 x 4.0 in.) flat Tomcat® sticky board (Motomco, Madison, WI) is stapled horizontally (flat) on the trap bottom and centered between the two solar-powered lights. The sticky board must lie flat to keep its sticky substance from sloughing off the board during hot days and therefore making it difficult to retrieve and view insects collected. The trap also consists of two rectangular pieces of preservative-treated wood (bottom and back), each measuring 50.5 x 13.8 x 2.0 cm (19.9 x 5.4 x 0.8 in.) and connected at a 90° angle, plus two rectangular support brackets, each measuring 13.9 x 3.2 x 1.8 cm (5.5 x 3.2 x 0.7 in.). Each pair of support brackets is connected at a 90° angle and affixed to each end of the back and base to complete the rectangular box. The ends and front of the box remain open and are covered with 19-gauge, 1.27-cm (0.5 in.) square aperture mesh hardware cloth to exclude large insects, birds, mammals, and debris. Semi-hemispherical Aquila® (ICP Global Technologies, Quebec, Canada) solar-powered "swimming pool" lights, measuring 14.8 cm in diameter x 9.8 cm (5.8 x 3.9 in.) tall, are self-contained and water proof. These lights are charged in direct light for 48 hours before being placed inside the trap, one at each end. A steel T-post was vertically driven into the ground and a single trap was attached with two U-bolts (Fig. 3.2c). Additionally, a smaller version of the Smith Light Trap (Figs. 3.3a, b), but without solar powered lights, measuring 30.5 x 13.8 x 2.0 cm (12 x 5.4 x 0.8 in.; bottom and back), was constructed for use under existing light sources. As with the larger light trap, for frontal support brackets each measuring 12 x 3.2 x 1.8 cm (4.7 x 1.3 x 0.7 in.) completed the box. The front and sides remained open and were covered with 19-gauge mesh, 1.27-cm (0.5 in.)-square aperture hardware cloth. This trap version also has a rectangular transparent acrylic top measuring 40.6 x 19 cm (19 x 7.5 in). This trap is attached to buildings and utility poles that already had sodium vapor lights in place (Fig. 3.3c). This trap houses only a sticky board. All monitoring devices were evaluated for trapped insects every three weeks.

Railroad crossties sold at home and garden centers and found along railroad tracks were regularly inspected during field evaluations. Additionally, county extension agents and pest control operators collected termite samples in glass vials containing 100 % ethyl alcohol (ETOH) previously provided to them. Each vial was labeled with the location and date collected.

Monitoring Sites. Monitoring devices were installed in March 2005 and monitored through October 2007. Monitoring sites were located in the southern Oklahoma counties of Jackson, Tillman, Cotton, Jefferson, Love, Marshall, Bryan, Choctaw, and McCurtain, all bordering the Red River on their southern boundaries (Fig. 3.4a). Monitoring sites were selected in cooperation with county extension agents and by approaching private landowners for permission to work on their properties. Each site was located in areas accessible at all times and where environmental factors were favorable for establishment of the Formosan subterranean termite. These factors included proximity to bodies of water, presence of trees favorable to the Formosan subterranean termite such as bald cypress, pine, and pecan, and adequate rainfall and temperature. The solar-powered and non-solar-powered traps were placed only on private property to avoid theft and vandalism. When public access land was used, the monitoring devices were concealed to avoid human tampering. Soil-surface ground-boards and in-ground monitoring devices were installed in all nine counties. Additionally, solar-powered light traps were installed in Love, Bryan, Choctaw, and McCurtain Counties. More attention was given to these four counties due to their relatively greater human population and commerce. These four southeastern counties have relatively higher humidity and rainfall, relatively lower ambient temperatures, and a greater abundance of standing trees compared with the five most southwestern counties noted. These conditions are ideal for Formosan subterranean termite establishment and proliferation. These four counties were also in closest proximity to the Texas counties that already have established Formosan subterranean termite populations. Counties west of Love County (Jackson, Tillman, Cotton, and Jefferson) have less annual rain fall, relatively higher temperatures, and fewer trees. Although the climate of these counties is not as conducive for the establishment of the Formosan subterranean termite, they were included for monitoring. Railroad crossties along railroad tracks and at home and garden centers were visually inspected in all nine counties. Additional visual inspections of railroad crossties were conducted from May through October at home and garden centers in Beckham, Carter, Comanche, Craig, Creek, Custer, Garfield, Oklahoma, Osage, Pittsburg, Payne, Rogers, Tulsa, Washington, and Woodward Counties (Fig. 3.4b). Termites were also collected from Cimarron, Kay, Kiowa and Payne Counties by pest control operators and colleagues, as well as from Osage County by Smith (2008).

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 a 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´-CGCCTGTTTATCAAAAACAT-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 1.5ml micro-centrifuge conical tube, the lid was closed and the tube was placed in liquid nitrogen for one-to-two minutes. The tube was removed from liquid nitrogen, opened and the termite(s) were pulverized inside the existing 1.5ml tube 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 K overnight 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 at the Oklahoma State University Biochemistry Microarray Core Facility. Any PCR product sample with < 10 ng/ul 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 100ml of TBE buffer and four 500mg 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. 5µl samples of PCR product were taken 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* and *C. formosanus* 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). The consensus sequences accession numbers are DQ001971.1, AY441992.1, AY257235.2, EU259775.1, and AY558911.1. 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.

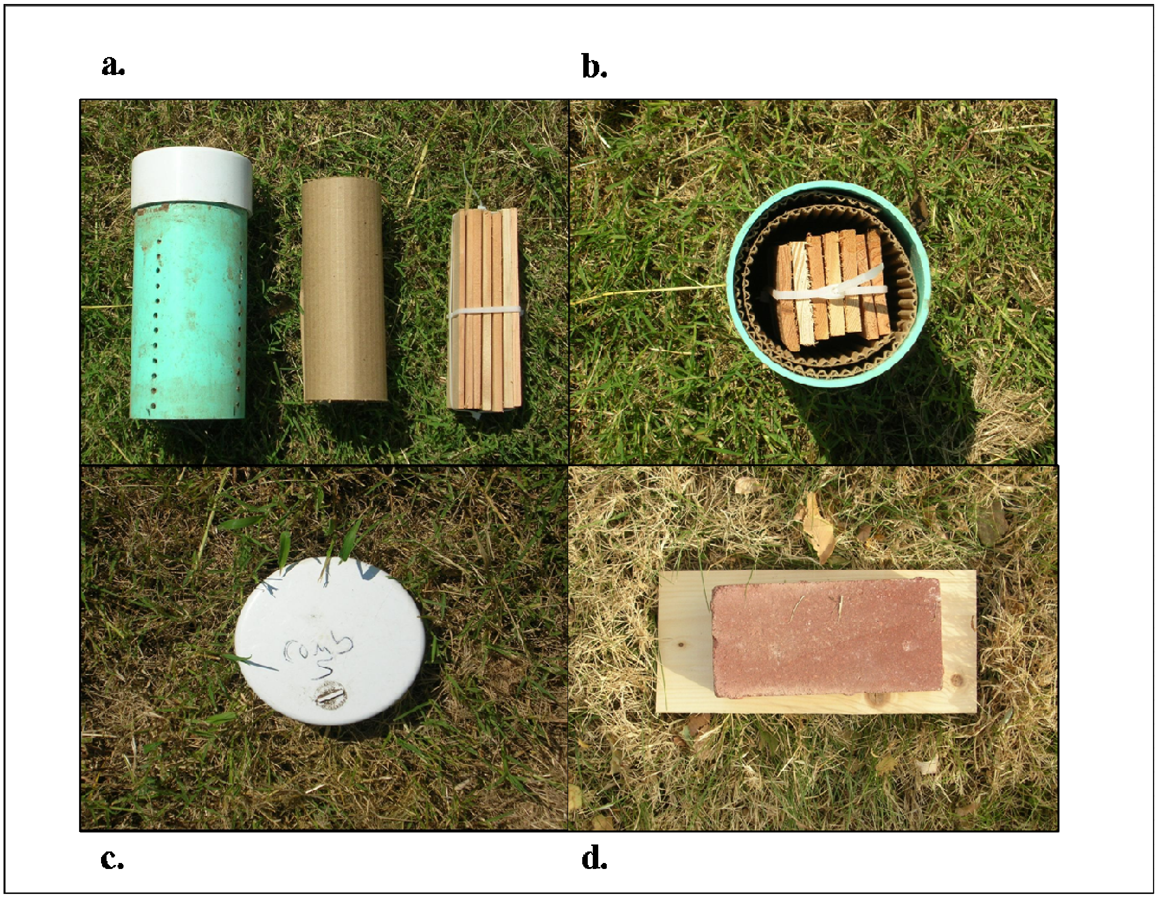

Fig. 3.1. (a) In-ground monitoring device components including PVC pipe, wood sandwich and cardboard, (b) assembled in-ground monitoring device (top view), (c) emplaced in-ground monitoring device with cap, (d) emplaced soil-surface ground-board.

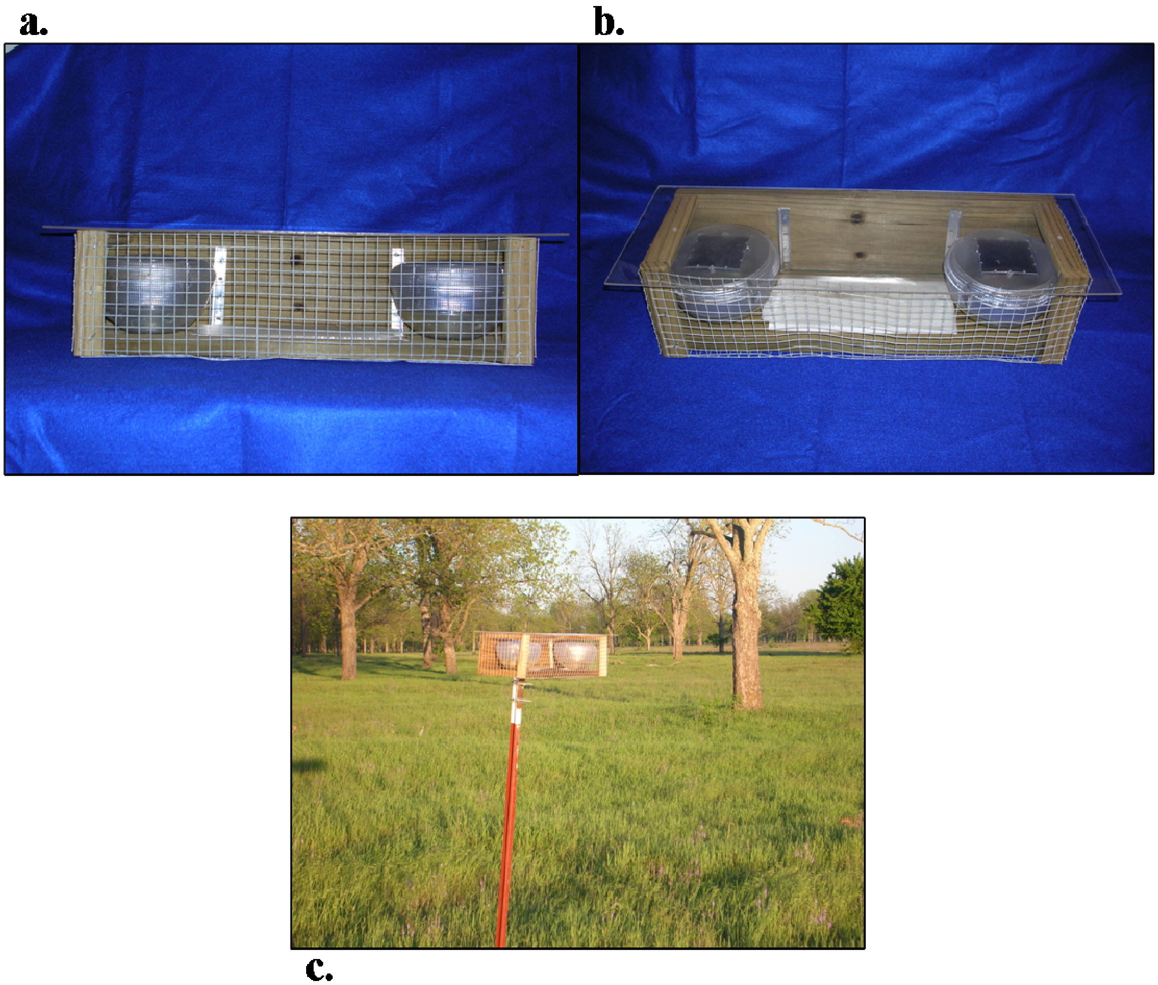

Fig. 3.2. Views of solar-powered Smith Light Trap: (a) frontal, (b) top, (c) emplaced.

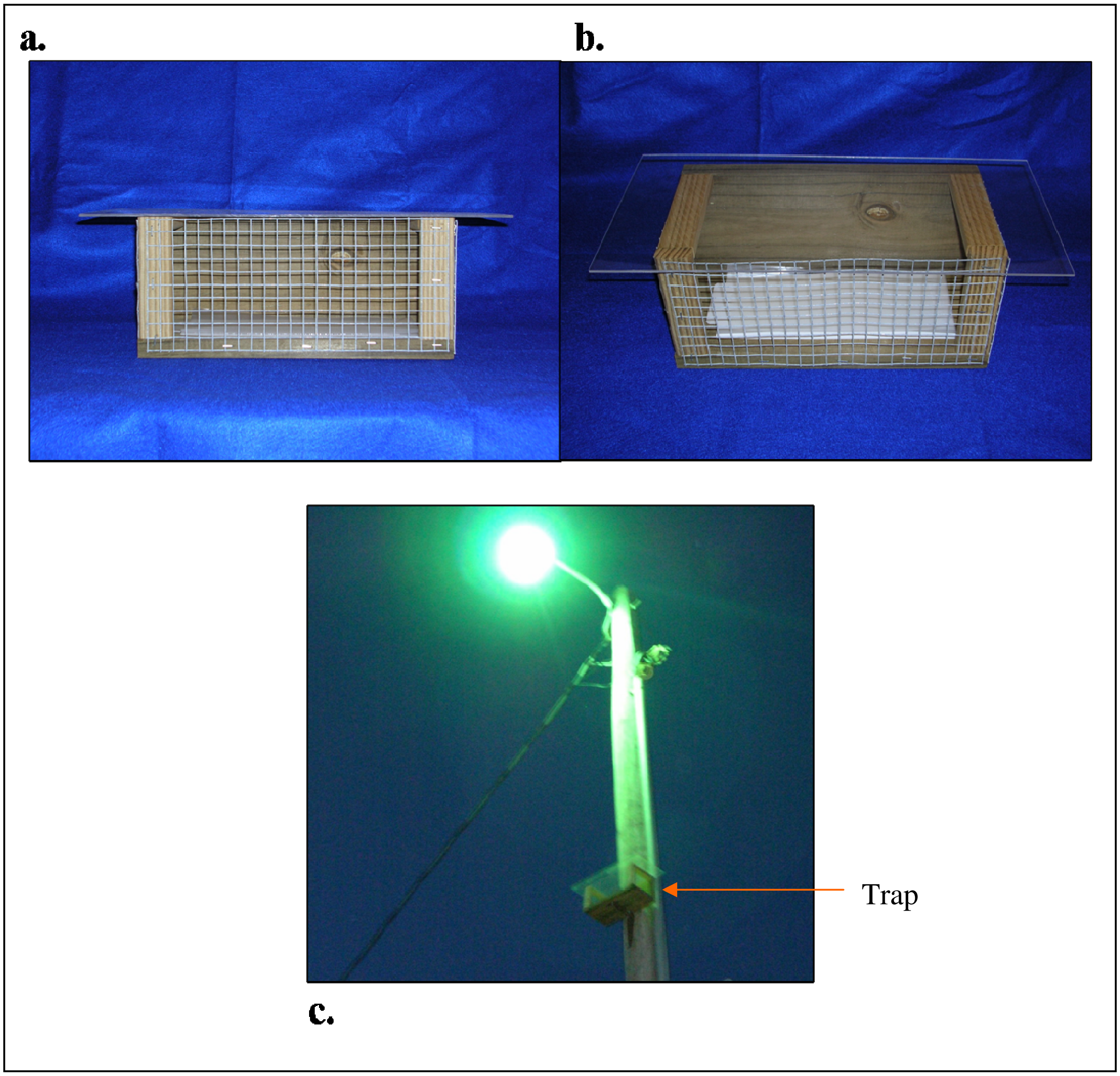

Fig. 3.3. Views of non-solar-powered Smith Light Trap: (a) frontal, (b) top, (c) emplaced.

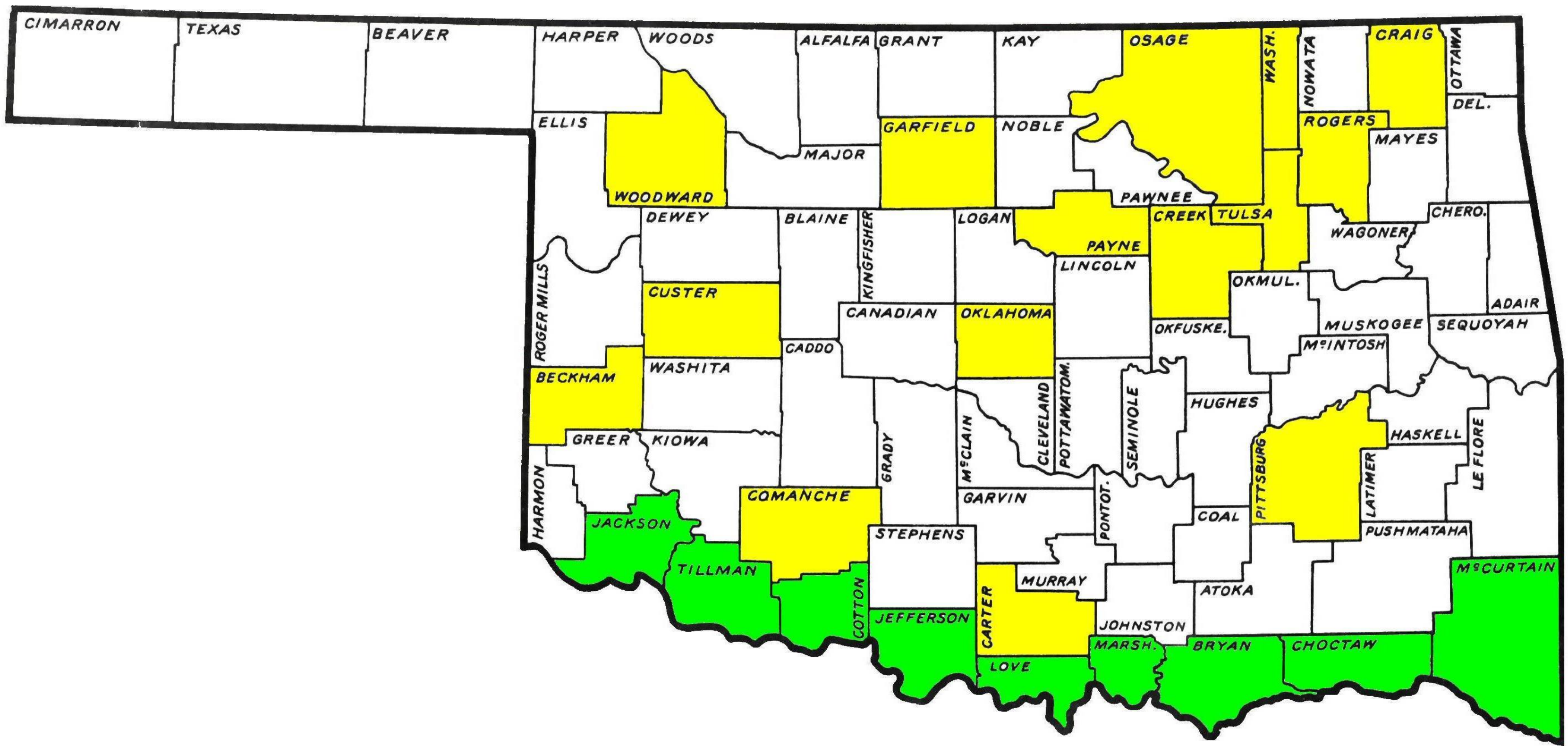

Fig. 3.4. (a) Oklahoma Counties shaded green contain monitoring devices for termite activity and received cyclic visual inspections. Railroad crossties stockpiled in home and garden centers, lumber yards and along railroad tracks were also inspected. (b) Oklahoma Counties shaded yellow received cyclic visual inspections of railroad crossties stockpiled in home and garden centers, lumber yards and along railroad tracks.

CHAPTER IV

RESULTS AND DISCUSSION

No Formosan subterranean termites were collected during the 2005 through 2007 survey. However, it may already be in Oklahoma. Due to the increasing number of reports of the Formosan subterranean termite moving northward through Texas, it is imperative to continue an on-going survey for this destructive pest within Oklahoma. Additional counties need to be added to the survey in conjunction with educational programs for pest control operators, extension agents, and homeowners on this impending destructive termite pest. It is only a matter of time before the Formosan subterranean termite becomes established in Oklahoma due to its continued rapid migration northward through Texas.

Termites endemic to Oklahoma were collected from eight different types of collection sites located in 25 cities and 14 counties (Table 4.1, Fig. 4.1). All morphological and molecular identifications of alates corresponded, with one exception; an alate morphologically identified as *R. virginicus* was molecularly identified as *R. flavipes*. *R. hageni* and *R.* tibialis soldiers could be positively identified morphologically. These identifications were verified molecularly. Samples containing *R. flavipes* and *R. virginicus* soldiers could not be positively identified to species, due to overlapping morphological characteristics. These two species could only be separated and positively identified using molecular techniques. Additionally, all samples containing only workers were molecularly identified to species. Three of the fourteen survey counties, Choctaw, McCurtain and Payne**,** overlapped with a previous survey conducted by Brown et al. (2004). The current survey of Oklahoma updated and expanded the known distributions of *R. flavipes, R. virginicus, R. tibialis* and *R. hageni* (Fig. 4.2). Additionally, *G. tubiformans* (Fig. 4.3a) was collected in Jackson County, 40 years after the first and only collection of this species in 1965 in Tillman County (D. C. Arnold, Oklahoma State University, pers. comm.). After four days of rain, *G. tubiformans* was collected from vegetation and cow manure covered with mud sheets produced by this termite (Figs. 4.3b, c).

All species except *G. tubiformans* were collected from wooden structures. The known range of *R. tibialis* is now expanded to include two new northern counties, Cimarron and Osage, and three new southern counties, Bryan, Cotton, and McCurtain. This species was collected from logs and in-ground monitoring devices located in open areas with direct sunlight. The known distribution of *R. hageni* was expanded to five new counties: Bryan, Kay, Love, McCurtain, Osage, and Payne. This species was found in shaded areas under soil-surface ground-boards, within in-ground monitoring devices, and in tree stumps. The least collected species was *R. virginicus*. It was collected from a wooden structure and under the bark of a dying tree (*Pinus* sp.). *R. flavipes* was the most abundant species collected and was found in soil-surface ground-boards, in-ground monitoring devices, logs and/or railroad crossties in 11 of the 14 counties surveyed.

Although no termites were found in railroad crossties examined at several home and garden centers, *R. flavipes* was found in a pile of old railroad crossties in the rail yard in the town of Marietta, Love County. Neither Formosan subterranean termite nor endemic termite alates were captured in solar-powered or sodium vapor illuminated light traps. Endemic Oklahoma termites are diurnal swarmers and were never caught on a sticky board. However, the light traps did attract and capture other insects such as mosquitoes, moths, beetles, crane flies, and leafhoppers. Additionally, a jumping spider (Salticidae) was found living in the base of at least one of the lights in almost every solar-powered light trap.

Environmental, human, and animal interaction influenced the placement of solar-powered light traps and in-ground monitoring devices. In-ground monitoring devices in McCurtain, Choctaw, and Love Counties occasionally became inhabited by red imported fire ants (RIFA), *Solenopsis invicta* Buren, or carpenter ants (*Camponotus* sp.). Invasion of monitoring devices by RIFA necessitated removal, cleaning, and relocation of the entire trap because fire ants are not easily removed. Soil-surface ground-boards were more effective in capturing termites in areas with dense RIFA populations as these ants preferred in-ground monitoring devices as nesting sites. In-ground monitoring device lids were regularly displaced at the Love County site by non-identified wildlife. The lids were placed back on the device and supplemented with a soil-surface ground-board, which was not disturbed. In June 2006, a solar-powered light trap was stolen from one McCurtain County site. This trap was replaced, and it plus another light trap were relocated to a less obvious, more secure location. On July 5, 2007, due to heavy rains the previous week throughout the state, the Red River flooded the areas where monitoring devices were installed in McCurtain and Love Counties. Four light traps, four soil-surface ground-boards, and four in-ground monitoring devices were lost as the soil washed away. These monitoring devices were not replaced due to continued high waters and impassable roads, and the research project was ending in three weeks.

Table 4.1. Species and corresponding collection sites by county.

County	City	Species	Collection Site	Collection Dates
Bryan	Cobert	*R. flavipes*	IGMD[a]	21-Jun-05
		R. hageni	IGMD	25-Jun-05, 06-Jun-07
	Durant	*R. flavipes*	IGMD	29-Mar-05, 01-Apr-05
	Mead	*R. tibialis*	log	05-Jun-07
		R. hageni	IGMD	21-Jun-05 (2)[b], 25-Aug-05, 14-Jul-06
Choctaw	Ft. Towson	*R. flavipes*	IGMD	21-Jun-05 (2), 25-Aug-05 26-Aug-05, 16-Mar-06 23-Jun-06, 13-Jul-06
		R. hageni	IGMD	21-Jun-05, 25-Jun-05 25-Aug-05 (2), 26-Aug-05, 26-Apr-07
	Hugo	*R. flavipes*	structure	27-Apr-07 (4)
	Sawyer	*R. flavipes*	SSGB[c]	26-Apr-07 (4)
Cimarron	Boise City	*R. tibialis*	log	07-May-07
Cotton	Walters	*R. flavipes*	IGMD	21-Jun-05
		R. tibialis	IGMD	26-Aug-05
Jackson	Eldorado	*G. tubiformans*	cow manure	19-Aug-05
		R. flavipes	IGMD	22-Jun-05
Jefferson	Waurika	*R. flavipes*	log	09-Oct-06
Kay	Ponca City	*R. hageni*	log	Oct-07
Kiowa	Synder	*R. flavipes*	structure	14-Nov-07
Love	Burneyville	*R. hageni*	SSGB	06-Jul-07
	Marietta	*R. flavipes*	railroad crosstie	27-Apr-07
Marshall	Madill	*R. flavipes*	IGMD	25-Aug-05

[a]In-ground monitoring device.
[b]Numbers in parenthesis indicate multiple colonies on the same date.
[c]Soil-surface ground board.

Table 4.1. continued. Species and corresponding collection site by county.				
County	**City**	**Species**	**Collection Site**	**Collection Dates**
McCurtain	Bethal	*R. hageni*	structure	Jun-05
	Broken Bow	*R. flavipes*	stucture	Jun-05 (3), Jul-06 (3)
		R. flavipes	log	01-Jun-06
		R. hageni	structure	Jul-05, Aug-06, Nov-06
		R. virginicus	structure	Jun-05
		R. virginicus	dying tree	15-Mar-06
	Hayworth	*R. flavipes*	SSGB	01-Jun-06
	Hochatown	*R. flavipes*	structure	24-Aug-05
		R. hageni	structure	Sep-06
		R. hageni	stump	24-Aug-05
	Idabel	*R. flavipes*	structure	Apr-05, Jun-05 (3) Jul-05 (2), Sep-05 (2) Aug-06 (2)
		R. flavipes	log	14-Mar-05
		R. hageni	structure	Jun-05
		R. tibialis	structure	Jun-05
	Smithville	*R. flavipes*	structure	Jun-05
	Valliant	*R. flavipes*	SSGB	26-Apr-07
		R. hageni	SSGB	26-Apr-07
		R. virginicus	structure	May-05
Osage	Foraker	*R. flavipes*	IGMD	29-Nov-07, 21-Dec-07
		R. hageni	IGMD	21-Dec-07
		R. tibialis	IGMD	29-Nov-07
Payne	Stillwater	*R. hageni*	structure	25-Aug-05
Tillman	Fredrick	*R. flavipes*	IGMD	21-Jun-05
		R. flavipes	structure	14-Nov-07

[a]In-ground monitoring device.
[b]Numbers in parenthesis indicate multiple colonies on the same date.
[c]Soil-surface ground board.

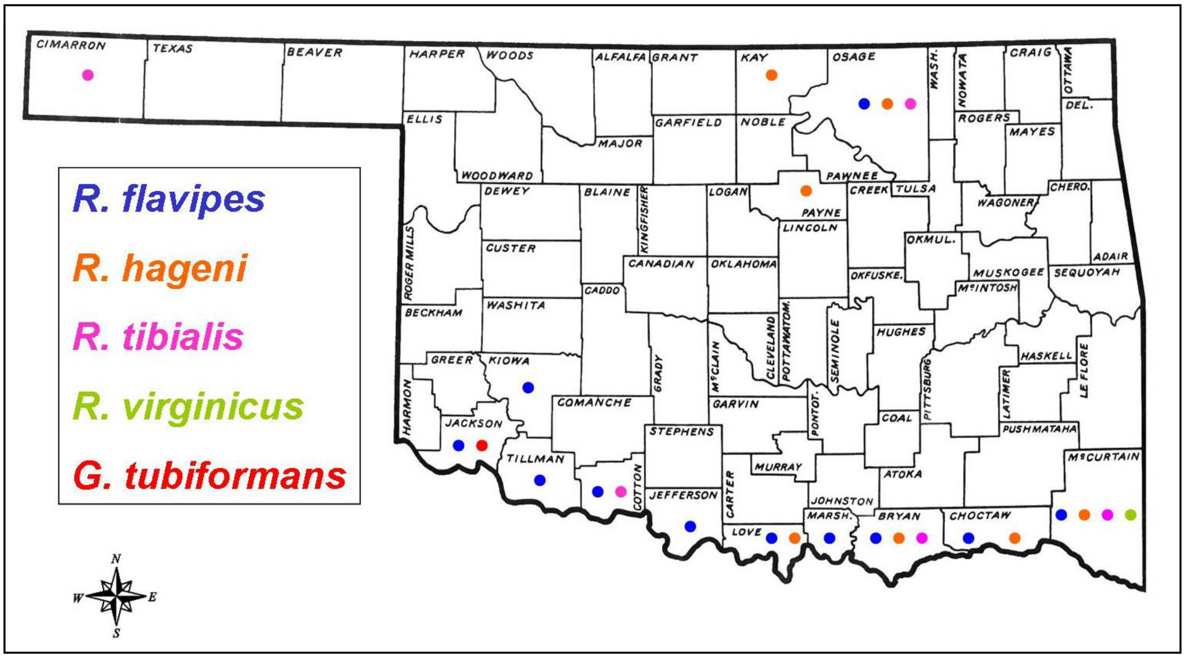

Fig. 4.1. Map of Oklahoma showing species per county for current survey.

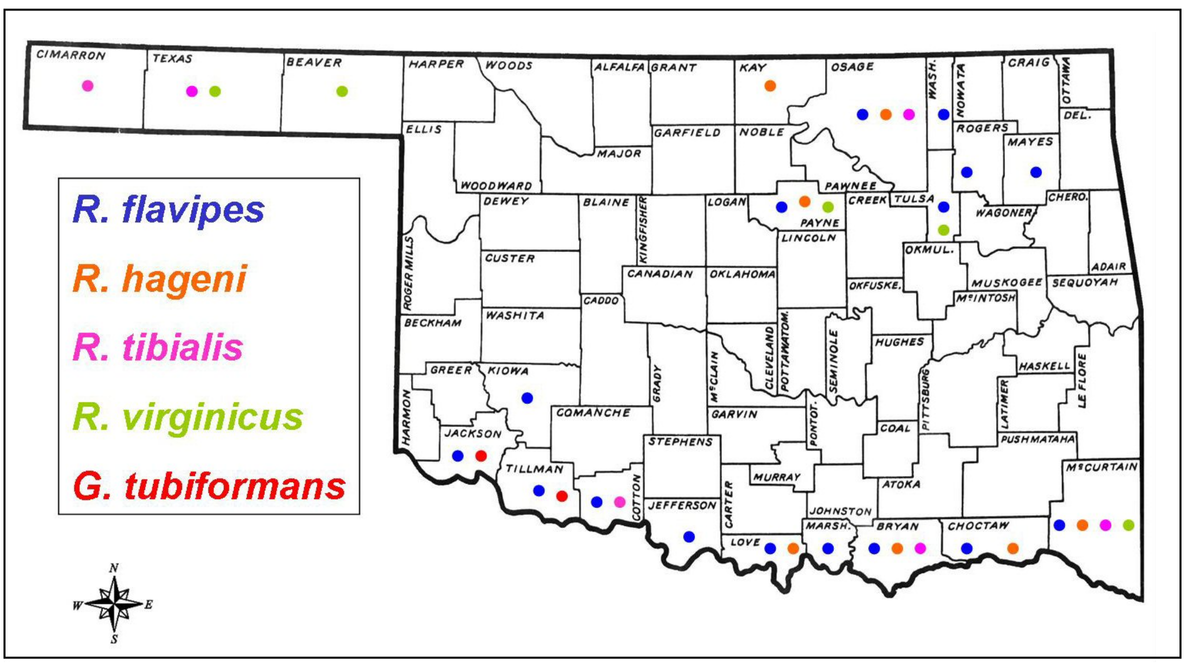

Fig. 4.2. Map of Oklahoma showing species per county for current and previous survey.

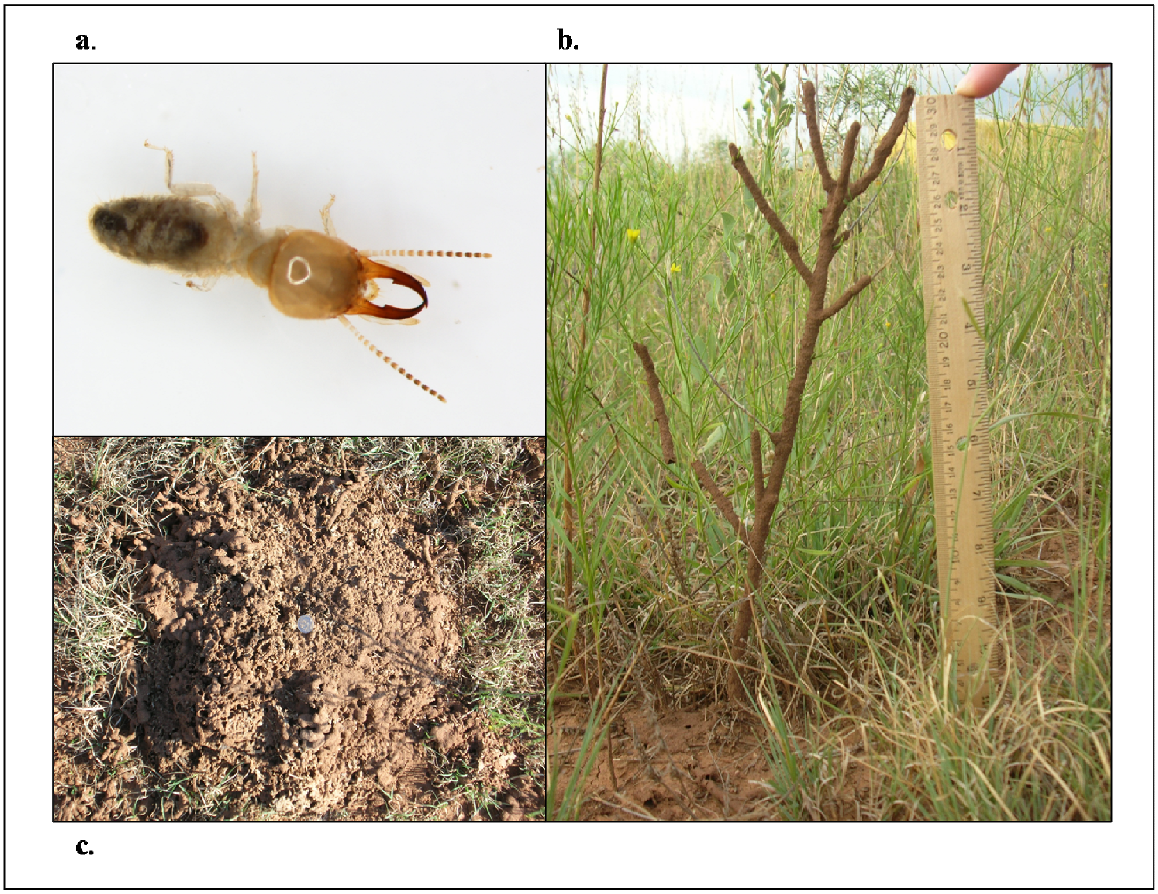

Fig. 4.3. (a) *Gnathamitermes tubiformans* soldier, (b) vegetation, and (c) cow manure covered with mud by *G. tubiformans.*

CHAPTER V

LITERATURE REVIEW

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VITA

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Candidate for the Degree of

Master of Science

Thesis: OKLAHOMA FORMOSAN SUBTERRANEAN TERMITE SURVEILLANCE PROGRAM AND TERMITE SURVEY

Major Field: Entomology

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Personal Data: Born in Tulsa, Oklahoma to Mike and Mae Miller. Married Matthew Paige Smith on October 8, 1993.

Education: Graduated from Ozark High School, Ozark, Missouri. Received Bachelor of Science degree in Entomology from Oklahoma State University, Stillwater in December 2004. Completed the requirements for the Master of Science degree with a major in Entomology at Oklahoma State University in May 2008.

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Name: Anita Lynn Smith Date of Degree: May 2008

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: OKLAHOMA FORMOSAN SUBTERRANEAN TERMITE SURVEILLANCE PROGRAM AND TERMITE SURVEY

Pages in Study: 46 Candidate for the Degree of Master of Science

Major Field: Entomology

Scope and Method of Study: The three objectives of this study were: 1) conduct a surveillance program for *Coptotermes formosanus* Shiraki, 2) concurrently conduct a central and southern county survey for endemic termite species, and 3) identify termites using morphological and molecular techniques. In-ground monitoring devices, soil-surface ground-boards, and elevated light-traps were installed throughout southern Oklahoma. Extension agents and pest management professionals also collected termite. Inspections of landscaping timbers and railroad crossties were conducted at commercial outlets for possible termite infestations.

Findings and Conclusions: *Reticulitermes flavipes* (Kollar), *R. hageni* Banks, *R. tibialis* Banks, *R. virginicus* (Banks) *and Gnathamitermes tubiformans* (Buckley) were collected, expanding the known distribution of these species. *C. formosanus* was not found although its eventual spread northward from Texas into Oklahoma appears certain. Termites were morphologically identified when alates and soldiers were available. Additionally, termites were identified and validated by DNA sequencing.

ADVISER'S APPROVAL: Bradford M. Kard