EFFECTS OF MULTIPLE GENERATIONS OF *Metarhizium anisopliae*

ON SUBTERRANEAN TERMITE FEEDING AND MORTALITY

A Thesis

by

KIMBERLY M. ENGLER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2004

Major Subject: Entomology

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Approved as to style and content by:

Roger E. Gold (Chair of Committee)	Kerry Harris (Member)
C.O. Patterson (Member)	Kevin Heinz (Head of Department)

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ABSTRACT

Effects of Multiple Generations of *Metarhizium anisopliae* on

Subterranean Termite Feeding and Mortality. (May 2004)

Kimberly M. Engler, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Roger Gold

This thesis evaluated the attractancy and mortality of *Metarhizium anisopliae* on two species of subterranean termites, *Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki. There were four specific objectives developed for this research. The first objective was to determine if *R. flavipes* or *C. formosanus* were attracted to the mycelium mat matrix of *M. anisopliae* cultured on rice or corn. The second objective was to determine the tunneling distances of *R. flavipes* and *C. formosanus* when exposed to aged strains of *M. anisopliae.* The third objective was to determine if the fungus caused mortality to populations of *R. flavipes* or *C. formosanus* in glass tube bioassays. The fourth objective was to determine if *R. flavipes* termites are attracted to an ethanol extract of mycelium of *M. anisopliae* (X-5) or a commercial preferred feeding product (Summon®)*,* and to estimate the percent consumption of the cellulose matrix. The extract and the Summon® disks were tested in the laboratory using glass plate bioassays, and in the field using commercial termite monitors containing each of the treatments individually.

The results with attractancy and mortality varied with age and generation of *M. anisopliae* mycelia*,* but all treatments were more attractive and caused more mortality than the controls*.* When presented with choices, both *R. flavipes* and *C. formosanus* did show preference to both the mycelium and the extract forms of *M. anisopliae.* The 1:1000 dilution of *M. anisopliae* extract (X-5) was strongly preferred over the other treatments, and all of the dilutions were

preferred over the Summon® and ethanol (40%) treated disks in the laboratory. An analysis of

the consumption of test cellulose matrix showed that Summon® did not attract termites, but it

was a phagostimulant. When the undiluted ethanol extract of *M. anisopliae* was tested in the

field, there were more termite visits to the ethanol extract of *M. anisopliae* (X-5) treated

monitors stations, and the fewest termite visits were observed in the monitors containing the

untreated fiber pulp disks.

DEDICATION

This thesis is dedicated to my family and friends. My parents, Bonnie and Kyle Engler, who have been extremely supportive, caring, optimistic, and motivational people in my life. They are the most generous and loving parents a person could ever have. They have taught me to love, laugh, and to be happy with my inner self. They told me that I could accomplish anything in life and to never accept mediocrity. I owe so much to them. To my grandmother, Evelyn Silvers, who I continue to owe much gratitude for helping out my family both financially and emotionally after a natural disaster. I admire her strength and ability to care so deeply for others. To my brother, Lee Taylor, for all of the support and advice through the years, and for giving me a "sister" and two nephews to adore. To all of my friends, some of which never understood why I liked science, but still continued to support me in all of my endeavors.

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INTRODUCTION

The order Isoptera contains approximately 2,700 termite species worldwide

(Nalepa, 2000). Termite species live in eusocial colonies comprised of different

castes. Each caste is distinguishable by its morphology and function within the

colony (Borror et al., 1992). For example, the primary reproductives are sexually

mature males and females and they fulfill the reproductive needs of the colony

(Moore, 1987). They accomplish this task through swarming flights to seek a new

nesting sites. Having arrived at a new site, they shed their wings, mate, and work

together to construct a new nest and colony (Borror et al., 1992). Therefore, the

entire colony is composed of descendents from one mating pair (Moore, 1987). The

winged reproductives are often the only caste that comes into contact with humans

(Figure 1).

The most numerous morphological forms within the colony are the workers.

Workers are immature males and females that are responsible for nest building and

repair, foraging, feeding, and grooming the other castes members. The soldier caste

is comprised of wingless, sexually immature males and females that defend the

colony from intruders. They accomplish this task either by using their large

mandibles to crush and dismember intruders or in some species, by releasing an

acidic, viscous fluid from a nasus (Borror et al., 1992). The immature termites,

known as pseudergates, may develop into reproductives, soldiers, or remain as

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This thesis follows the format of the Journal of Economic Entomology.

workers depending upon environmental and social factors within the colony

(Krishna, 1969).

a.   b.
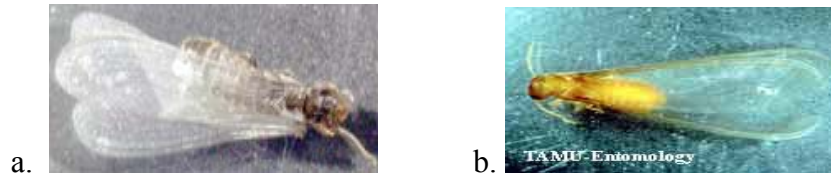
Figure 1. (a) An alate of the species *Reticulitermes flavipes*. (b) An alate of the species *Coptotermes formosanus*.

Termites obtain nutrients by breaking down cellulose in their digestive tract.

Their nutritional metabolism is referred to as xylophagy or wood-eating. Termites

benefit our ecosystem by converting cellulose into usable nutrients. However, they

become serious economic pests when they colonize and damage wooden human

dwellings and structures (Thorne, 1998). In the family Rhinotermitidae, there are

two economically important subterranean termite pest species in the United States:

the Eastern subterranean termite, *Reticulitermes flavipes* (Kollar) and the Formosan

subterranean termite, *Coptotermes formosanus* Shiraki. The presence of termites in

an area is favored by specific temperature, humidity, and soil moisture and type.

Subterranean termites are found in every state in the United States except Alaska,

and the relative number of termite infestations decreases as one moves north

(Nalepa, 2000). At least 2 billion dollars is spent annually for controlling termites

and repairing damage caused by these insects. Subterranean termites are responsible

for 80% of this damage. *R. flavipes,* by far the most abundant and widespread species (Cornelius and Osbrink, 2001), is found throughout North America. Whereas this species prefers tropical or subtropical climates, it is found as far north as Kincardine, Ontario where it causes serious economic damage (Grace et. al., 1989).

C. formosanus is thought to have entered the United States more than fifty years ago in wooden shipping crates from China. High humidity and moderate temperatures have allowed the Formosan termite to spread coast to coast in the Southern United States (Wright, 2002). In a colony to colony comparison, *C. formosanus* normally causes more economic damage than *R.flavipes*, due to its aggressive behavior, greater feeding rate, greater variety of materials attacked, larger foraging territories, and larger colony sizes (Cornelius and Osbrink, 2001).

Both termite species live in nests composed of wood, soil, and excrement (Rosengaus et al., 1998). Subterranean termites live in wood buried beneath or in contact with the soil but maintain passageways or connecting gallery to the soil in order to obtain moisture (Lai et al., 1982). Colony survival depends on the construction of tunnels and galleries in the soil. The galleries allow the colony to come into contact with food resources and to regulate environmental conditions according to their needs (Ettershank et al., 1980). *R. flavipes* must maintain a ground connection to obtain adequate moisture (Borror et al., 1992). The water needs of the Formosan subterranean termites are similar to those of *R. flavipes;* however, *C. formosanus* can establish and maintain aerial colonies that do not have contact with

the soil (Wright, 2002). *C. formosanus* nests are composed of cartons, partially digested wood glued together with feces and salivary secretions, which are porous and allow water storage for the aerial colony (Jones and Howell, 2000).

Both of these subterranean termite colonies provide a controlled microclimate, due in part to limited exchanges of air with the outside environment. The humid microclimate within the nest is ideal for fungal growth. Many strains of fungi normally occur within the nests, where they compete with the termites for plant remains. In fact, these subterranean termites generally prefer fungus-infested wood because the fungi partially decompose the cellulose and lignin into a more digestible form. Over time, relationships between some termites and fungi have evolved into symbiotic ones (Sands, 1969).

Some fungi can be toxic or pathogenic to insects. Fungal diseases often contribute significantly to the natural mortality of many insect populations, because fungi can penetrate the insect's cuticle (Ferron, 1978). The time of heightened metabolic activity prior to sporulation in fungi is likely when attractancy of the fungi to insects is greatest (Kramm et al., 1982). At this time, foraging termites usually become contaminated with fungal spores and subsequently transport the spores to the colony (Hoffman et al, 1993). Once the spores attach to the host, they may be deposited within the colony.   This horizontal transmission occurs when a pathogen is transferred from one individual to another. Once the spores are in the colony, they can be spread by: 1) mutual exchange of nutrients between colony members, called trophallaxis; 2) grooming, which consists of termites licking the body surfaces of

nest mates; and, 3) cannibalism, which regulates the number of individuals in each caste and recycles nitrogenous nutrients (Kramm et al., 1982). Thus infected termites can rapidly spread a fungal pathogen to healthy termites, greatly increasing the incidence of the disease agents and mortality within the colony (Zoberi and Grace, 1990). In general, fungi are excellent candidates for use in integrated pest management programs because they do not need to be ingested to cause infection and their activity can be enhanced with the production of cuticle degrading enzymes (Wright et. al., 2002).

The effectiveness of entomogenous fungi as biocontrol agents can be increased by manipulating environmental factors or by artificially introducing an inoculum of the pathogens into the termite population (Zoberi, 1995). The fungus, *Metarhizium anisopliae,* is a promising biological agent for controlling termites.

It is a commonly found pathogen in nature and is in the Phylum Deuteromycota (imperfect fungi) which infects over 200 insect species, including termites at all lifestages. The fungus is easily identified because it forms green chains of cylindrical conidia that densely compact on the infected host, causing green muscardine disease (Zimmerman, 1993). The infection process begins when fungal spores or conidia land on the cuticle of a susceptible insect (Gillespie et al., 2000). The conidia may persist in the soil in a dormant stage for three or more years or until initial contact with the termite cuticle induces germination (Milner, 2003). A complex of specific interactions, such as enzymes, and nonspecific interactions, such as hydrophobicity, are responsible for the spore-cuticle interaction that mediates

attachment. The rodlet layer of the spores come into contact with the host's

epicuticle, and the topography and chemical properties of the epicuticle enhance

adhesion of the spore and help to orientate the germ tubes into the cuticle (Cole and

Hoch, 1991). Sporulation is followed by penetration of the host's cuticle, usually

between the mouthparts, at inter-segmental folds, or through spiracles. These areas

have a high level of moisture that promotes germination (Zimmerman, 1993). The

fungal spore uses a combination of mechanical pressure and cuticle degrading

enzymes, such as lipases and proteases, to attack and dissolve the cuticle (Khetan,

2001).

 After the fungus penetrates through the cuticle, it invades into the hemocoel to

form a dense mycelial growth (Zimmerman, 1993).   As the mycelium penetrates

through the host, *M. anisopliae* produces toxins known as destruxins and

cytochalasins (Wright et al., 2000). Destruxins are produced as the mycelium grows

inside the insect. Destruxin forms A and B depolarize muscle membranes by

activating calcium ion channels, which leads to lifelessness and paralysis.

Destruxins C and E are immunosuppressive and cytopathic (Boudas, 1998). The

toxin cytochalasins acts to block actin filament elongation. The life cycle of the

fungus is completed when it sporulates on the cadaver of the host. The external

hyphae produce conidia that ripen and are released into the environment. This

allows horizontal transmission of the disease within the termite colony (Khetan,

2001).

In order to understand the full potential of *M anisopliae's* epizootic potential, its virulence and infection methods, as well as culture methods and formulations must be investigated (Lacey et al., 2001). Horizontal transmission of fungi is an important component in controlling termites, due to subterranean termites living in remote areas that are hard to treat. Horizontal transmission is the basic idea behind commercial termite monitoring systems for termites. In a monitoring system, the active ingredient is impregnated into a cellulose substrate housed in a plastic monitor station. These stations are then placed in the ground where termites can discover and consume the cellulose (Su et. al., 1987). However, in order for the termite monitoring system to function properly in the environment, the fungal isolate must not be highly pathogenic or a learned avoidance response may occur. The idea behind a baiting system is to target the whole termite colony with slow-acting, non-repellant chemicals or disease agents that will be distributed to the colony by foragers through social interactions (Su et. al., 1987).

There are numerous advantages when using fungi as biocontrol agents, such as relative safety to humans and other non-target organisms, reduction of pesticide usage, and increased activity of natural enemies (Lacey et al., 2001). Fungal biocontrol agents can be improved by genetically altering the fungus' efficacy, stability and marketability (Milner, 1991). Therefore, multiple generations of *M. anisopliae* need to be tested to select for traits that increase its effectiveness as a biocontrol agent. There are three characteristics of *M. anisopliae* that can be altered genetically: early germination, early sporulation, and enhanced destruxin production

(Al-Aidroos and Roberts, 1978). In one published study, the genes responsible for toxin production were amplified to expand host range and enhance pathogenicity (Gardner and McCoy, 1992). It has also been demonstrated that nonvirulent strains of *M. anisopliae* can be rendered virulent by long term, sequential culturing of the fungus in insects (Milner, 1991). Sequential culturing results in increased levels of both fungal attractants and pesticidal metabolites through nutrient loading techniques. Selecting and reisolating spores from inoculated insects that die soon after exposure produces more virulent strains of *M. anisopliae* (Heale, 1988). In another study, it was suggested that successive passage through a host insect selects for enhanced germination of conidia, thus increasing the capacity of the fungus to cause infection (Daoust et al., 1982).

OBJECTIVES

There were four specific objectives developed for this research project. The first objective was to determine if the Eastern subterranean termite, *Reticulitermes flavipes* (Kollar), or the Formosan subterranean termite, *Coptotermes formosanus* Shiraki were attracted to the mycelium mat matrix of *Metarhizium anisopliae* cultured on rice or corn. Termites have an endless number of cellulose food sources, so attracting them to a certain area using fungi may help to control their populations.

The second objective was to determine the tunneling distances of *R. flavipes* and *C. formosanus* when exposed to aged strains of *M. anisopliae* in bioassay tests. This would help to determine if the mycelia lose efficacy through time, when placed in a controlled environment.

The third objective was to determine if the fungal mycelia cause mortality in *R. flavipes* or *C. formosanus* colonies in bioassay tubes. This would help determine if the fungal spores are virulent and effective in the control of termite populations.

The fourth objective was to determine if *R. flavipes* termites are attracted to an ethanol extract form of mycelium of *M. anisopliae* (X-5) and, a commercial, preferred feeding product (Summon®). Estimates were also made of the percent consumption of the cellulose matrix in these treatments. The extract and the Summon® were tested in the laboratory using glass plate bioassays, and in the field using commercial termite monitors.

MATERIALS AND METHODS

R. flavipes used in the experiments were collected from sites in Bryan and College Station, Texas. They were collected in a trapping mechanism consisting of Polyvinylchloride (PVC) that were 12.5 cm in height and 10 cm in diameter filled with moistened corrugated cardboard (Figure 2).

Figure 2. *Reticulitermes flavipes* trap made up of 12.5 X 10 cm PVC pipe filled with moistened strips of corrugated cardboard.

C. formosanus were collected from Texas City, Texas. These termites were trapped using three 12x12 cm long wooden boards that were linked together using a 14 cm screw and metal washers in between each board (Figure 3). These wooden traps were moistened with water and placed within a plastic bucket, with the bottom removed, in the ground and sealed with a lid on top of the bucket. The plastic bucket was 16.5 cm

tall and 18.4 cm in diameter. The wooden traps remained in place for a month, or until

termite activity was noted.

Figure 3. *Coptotermes formosanus* trap made up of three 12x12 cm wooden boards that were placed on a 14 cm long screw and secured at the end by a wing nut.

After activity was detected in the cardboard and wood traps, termites were brought to

the laboratory to be sorted from the cardboard or wood. These termites were maintained

in Falcon 150x25 mm sterile plastic petri dishes in the laboratory where they were fed

moistened Fisher brand tongue depressors (Figure 4). Both species of termites used in

the experiments were held in the laboratory for less than two months.

Figure 4. *Reticulitermes flavipes* in a Falcon 150 X 25 mm sterile petri dish with Fisher brand tongue depressors used as a source of cellulose and harborage.

    Metarhizium anisopliae was tested in two forms. One form was the mycelium mat matrix cultured on rice or corn, and the other was a 40% ethanol extract of *M. anisopliae* (X-5) cultured on rice. The exact ethanol extraction procedures are proprietary and were developed by Paul Stamets of Fungi Perfecti in Shelton, WA. He provided both the mycelia and ethanol extracts for these studies. Upon arrival each sample was logged-in and either stored in a refrigerator at 4° C or held in the laboratory at 24°C until the time of testing in individual plastic containers. The tests evaluated both substracte attractiveness and termite mortality caused by aged fungal mycelia at 1, 2, 4, 8, and 12 weeks, respectively.

    Paul Stamets cultured subsequent generations of the fungus by transferring mycelium from one culture dish to another and allowing the cells to multiply. One generation of *M. anisopliae* was cultured on rice and two generations were cultured on corn. They were tested in an olfactometer and glass bioassay tubes. The ethanol extract of *M. anisopliae* (X-5) was tested in the olfactometer, glassplate bioassays, and in a termite monitoring system.

Olfactometer: In one set of experiments, an olfactometer was constructed (Williams, 2001) and used to evaluate volatiles produced by fungal strains. This was done to quantitate the attractancy of these volatiles to the termites. The olfactometer (Figures 5-6) consists of a main 5 cm diameter PVC pipe that was octagon in shape with 55.9 cm sides that were connected in eight places with 5.1 cm joints 10.2 cm in length. The main PVC pipe had a total diameter of 127 cm that was raised by 30.5 cm wooden boards, so the main PVC pipe was above all of the petri dishes positioned below. The purpose of the main PVC pipe was to serve as a reservoir for air used in all of the radiating petri dishes. A Cole-Parmer Model No. L-79200-00 pump was connected to the 5.1 cm PVC pipe with flexible 1.0 cm ID PVC tubing, which allowed 2.5 ml/min of outside air to enter into the main PVC ring. The pump was placed outside the treatment chamber in order to avoid excess vibration to the platform that could alter the natural behavior of the termites. The pump was run day and night for the length of the experiment. Each of the six test chambers consisted of a center Falcon 150 X 25 mm sterile petri dish with two 5.1 cm pieces of glass tubing attached to the upper end of the dish to serve as a release valve for excess air. At the lower end of the center dish, six radiating Fisher brand 100 X 15 mm sterile petri dishes were connected with 10.2 cm glass tubing to the center dish (Figure 6). Each of these radiating dishes contained either a treatment of *M. anisopliae* mycelia that had been refrigerated (4° C) or ethanol extract of *M. anisopliae* (X-5), controls of sterile rice or corn, or untreated cardboard made from FC kraft pulp disks produced from 100% Douglas Fir in Samoa, California. The wooden platform for the olfactometer was 182.9x182.9 cm that

accommodated, six center dishes and thirty-six radiating petri dishes. In order to allow

equal airflow across the thirty-six treatments in the radiating dishes, a 55.9 cm (length)

of 1.00 cm ID flexible PVC tubing was attached from the main PVC pipe by Fisher

brand disconnects to a Cole-Parmer Model No. PMR 1-010265 aluminum flow

controller. The flow controller allowed the appropriate volume of air to flow into each

test chamber. The incoming air was regulated to 1.12 ml/min. The air flowed out of

the flowmeter into 10.2 cm (length) of 1.00 cm ID flexible PVC tubing connected to a

Gelman AquaPrep filter that removed particles 0.5 μm and larger. These filters

prevent outside contaminants from entering the closed apparatus. The filtered air then

flowed through a 35.6 cm (length) of 1.00 cm ID flexible PVC tubing and into each

radiating dish (Figure 5).

Figure 5. Olfactometer design used to determine the termites' preferences for volatiles produced by the *M. anisopliae* fungal strains.

Figure 6. A view of one replication found in the olfactometer design that was made up of a center chamber and the six radiating dishes.

The olfactometer evaluated the attractancy of both the mycelia mat matrix on corn or rice, and the fungal extract (X-5). When termites were placed in the apparatus, they orientate and move towards the most attractive odor source. The first generation of *M. anisopliae* cultured on rice and the second generation of *M. anisopliae* cultured on corn were tested in the olfactometer.   Testing involved aging the strains of fungi cultured on the rice or corn for a maximum of 3 months in the refrigerator (4° C), and testing the attractancy of the fungi as it aged at 1, 2, 4, 8, and 12 weeks, respectfully. The mycelia treatments placed into the radiating dishes were eight corn kernels or sixteen pieces of rice, respectively. The control treatments involved the placement of eight corn kernels or sixteen pieces of rice into the radiating dishes, respectively.

When *R. flavipes* also were used in the olfactometer to also test the attractancy of ethanol extracted mycelium of *M. anisopliae* and Summon® (FMC, 2003).   The

treatments used in these tests were ethanol treated fiber cement kraft pulp disks, *M. anisopliae* extract treated disks, and Summon® disks. The disks were emersed into their appropriate testing treatment, and then allowed to dry for 8 hours. For the olfactometer experiments a total of 300 termites from each species, with one soldier for *R. flavipes* and two soldiers for *C. formosanus*, were placed separately in the center dish at the time of initiation. Tests were conducted separately for each termite species. Termites were allowed to forage randomly into the radiating dishes (Figure 6). The apparatus was placed in a separate room to reduce the possible contamination by spores in the ambient air. Counts of termite preference were made hourly for the first 5 hrs and then daily for two days. The light in the room was kept on day and night (L:24 hrs.). The apparatus allows three replicates of each species to be evaluated in each trial.

Glass Tube Bioassays: Glass tube bioassays allow mortality and attractancy to be evaluated. The bioassay tubes are similar to those described by Su *et al.*, 1993; Gold *et al.*, 1994; Gold *et al.*, 1996; and Waite *et al.*, *In Press*. The total length of the glass tubes was 85 mm. The treated tubes contained a 10 mm top layer of agar, followed successively by a 15 mm layer of soil, a 35 mm layer of *M. anisopliae* impregnated rice or corn, a 15 mm layer of soil, and a 10 mm bottom layer of agar. Two types of control tubes were used. The first contained a 10 mm top layer of agar, followed by a 15 mm layer of soil, a 35 mm layer of sterile rice or sterile corn, a 15 mm layer of soil, and a 10 mm bottom layer of agar. The second control type contained a 10 mm top layer of agar, 65 mm of soil, and a 10 mm bottom layer of agar. The bottom of all the tubes contained a 3 cm piece of wooden applicator stick, and both ends of the glass tube were enclosed

by foil and a rubber band (Figure 7). The soil used in the bioassay tubes was comprised

of 100 g of soil from College Station, Texas mixed with 10 ml of water.
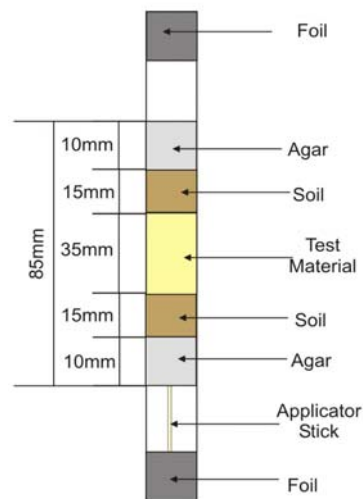
Figure 7. Bioassay tube set-up to measure tunneling distance and mortality of 30 *Reticulitermes flavipes* or 30 *Coptotermes formosanus* after 6 days of exposure to the treatment. Termites were placed at the top of the tube in between the foil and agar and allowed to tunnel vertically.

The mycelia used in these bioassays were the first generation of *M. anisopliae*

cultured on rice, and the second generations of *M. anisopliae* cultured on rice and corn

(Figure 8). Groups of 30 termites from each species, including one soldier for *R.*

flavipes and two soldiers for *C. formosanus*, were tested separately by placing them on

top of the agar in each tube and allowed to tunnel vertically through the tube. The

bioassay tubes were maintained in an environmental chamber to regulate temperature

at $25 \pm 2°C$ and humidity. The tunneling distance was observed after a period of six

days. On the sixth day, the tubes were carefully disassembled and mortality of the termites was determined. Three replicates of each treatment in the bioassay tubes were made for each of the two termite species.

Figure 8. Bioassay design used to test the tunneling rate and mortality of both termite species when exposed to *M. anisopliae* grown on rice and corn.

Glass Plate Bioassay Studies: Glass plate bioassays were set up in laboratory to determine *R. flavipes* recruitment and percent consumption of the cellulose matrix in each treatment. Recruitment is defined as one termite finding a preferred treatment that caused the redirection and movement of nest mates to the preferred treatment. The experimental design followed similar designs used by N.Y. Su and H. Puche (2003) and Robson et al. (1995). The nest was defined as the center petri dish on the upper glass plate. Two glass plates, each measuring 35.6 X 35.6 cm, were used in this experiment. The two glass plates were centered over each other, with a 0.3 cm gap between them. On all of the sides of the square, a 2.5 cm wide glass strip that was

35.6 cm long and 0.3 cm in height serves as a barrier to prevent termites from escaping

from the treatment areas. In order to form a 0.3 cm amount of thickness between the

two glassplates, two FC kraft pulp disks for each treatment were glued together with

Elmer's All Multipurpose Glue. The glued disks were allowed to dry for 24 hrs, in

order to avoid altering the termites' natural foraging behavior. In the glass plate study,

five replicates were made for each treatment including: dried pre-weighed untreated

fiber cement kraft pulp disks; ethanol extract of *M. anisopliae* (X-5) treated fiber

cement kraft pulp disks; 40% ethyl alcohol treated FC kraft pulp disks; and Summon®.

Each disk was approximately 3.5 cm in diameter. There were four different dilutions

used for the ethanol extract of *M. anisopliae* (X-5) treated disks including, 1:0

(undiluted), 1:10, 1:100, and 1:1000. To prepare each dilution, the full strength 40%

ethanol extract was diluted with ethanol, to make the desired concentrations. For

instance, when making the 1:10 dilution, 10 ml of X-5 shipped from Fungi Perfecti

was combined with 90 ml of 40% ethanol in a plastic 237 ml Fisherbrand wide mouth

bottle. This solution was then inverted to ensure thorough mixing. All of the FC kraft

paper disks were soaked in the test solution until they were completely saturated, and

then the disks were dried in a fume hood for 8 hrs.

The upper glass plate had five holes drilled through the glass. A single center hole

was drilled 17.8 cm from the edge of the glass plate. The other four holes were 7.6 cm

from each of the four corners. A Falcon 150 X 25 mm sterile petri dish with a 0.3 cm

hole burned in the middle of the dish was attached to the middle of the upper glassplate

with silicone. The treatments were placed in all four corners within the glassplates,

and sifted College Station soil, which was free of rocks and other debris, was placed in between the two glassplates. The soil was funneled into the glassplates, until the inner 30.5 X 30.5 cm square was full of soil. On all sides, clamps were always used to keep the plates together. The plates were then laid flat on a table and 4 ml of distilled water was added through each hole to moisten the soil.

Approximately five hundred termites (1.5 g) were placed in each of the petri dishes attached to the center of the upper glassplate. Termites were placed in the petri dish along with one tongue depressor cut in half and placed on each side of the petri dish. Termites were allowed to tunnel normally from the center petri dish into the soil between the two glassplates to the treatments found in the four corners. Termite foraging was observed, so preference between the treatments could be determined in this experiment. Once the termites had tunneled to the treatments, daily counts of termites feeding on each treatment were taken daily for 12 days. Every other day, 4 ml of water was added to each of the four holes around each treatment to provide adequate moisture to the foraging termites.

Digital pictures were taken daily of each glassplate, to document the number of termites in each treatment (Figure 9). After 12 days, the glassplates were diassembled and the treatment disks were removed. The disks were placed in an oven and dried for 4 hrs. At the end of 4 hrs, the disks were allowed to cool, and then were reweighed. Comparisons were then made to the pre-testing weight to determine the amount of each disk that had been consumed by the termites. There were a total of 20 replications of the glassplate bioassays.
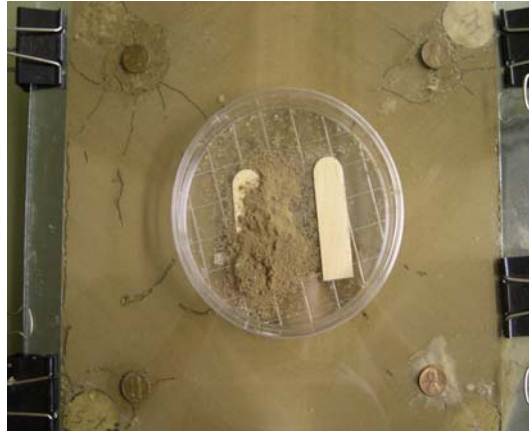
Figure 9. Replicate 1 of the undiluted X-5 treatment at day 10 in the glassplate bioassay study.

Field Evaluation: A field plot was established in Bryan, Texas in order to test the attractancy of *M. anisopliae* extract on the native subterranean population of *R. flavipes*. The field plot was 4.6 X 6.4 m. The field plot was monitored twice a week for 8 months, to determine the areas with active termite populations. The experimental design is similar to that used by Houseman *et al.* (2001). The Whitmire® monitoring system was used, with four monitors placed 30.5 cm from each other at each of the five active sites in the field plot.

The five active sites within the plot were then selected for evaluation. The attractancy of the ethanol extract of *M. anisopliae* (X-5) treated fiber cement kraft pulp, and Summon® made by FMC Corporation were evaluated in this study. Four treatments were used that included untreated fiber cement kraft pulp, 40% ethyl alcohol treated fiber cement kraft pulp, ethanol extract of *M. anisopliae* (X-5) extract treated fiber cement kraft pulp, and a Summon®. The fiber cement kraft pulp sheets

were cut into 3.5 cm circles that were the same size as the Summons®. The undiluted ethanol extract of *M. anisopliae* (X-5) and 40% ethyl alcohol fiber cement kraft pulp disks were immersed in their respective treatment and then left under the fumehood to completely dry. After the treatments dried, they were added individually to the bottom of each Whitmire® monitor.

The same treatments evaluated in the field plot were also tested around urban structures with active termite infestations. A total of 304 commercial Termitrol® monitoring stations were installed around 5 structures. The monitors were installed around the perimeter of the structures in clusters of four and each cluster was approximately 3 m apart. In the Termitrol® stations, the wooden monitor was replaced with a 12.7x20.3 cm sheet of fiber cement kraft pulp cardboard cellulose matrix. The treatments were: an untreated disk, ethanol treated disk, 1:0 (undiluted) ethanol extract of *M. anisopliae* treated disk, or Summon®. One of the treatments was randomly assigned to one of the four monitors in each cluster using a statistical calculator. Readings were taken twice a week for 17 weeks to determine the presence or absence of termites in the monitoring systems. Twice a week, 4 ml of undiluted ethanol extract of *M. anisopliae* (X-5) and 4 ml of 40% ethanol was added to the appropriate station on the FC kraft pulp disk located on the bottom of the station. Summon® was replaced through time as needed.

RESULTS

Olfactometer: The summation of the olfactometer testing with *C. formosanus* and *R. flavipes* are presented in Figure 10. In these tests, three hundred termites of each species were used in each replication for each test; however, *C. formosanus* foraged significantly more ($p \leq 0.05$) than did *R. flavipes* from the central nest chamber to the radiating dishes containing the treatments. The mean number of *C. formosanus* and *R. flavipes* that moved from the center dishes to the treatment chambers were 12.7 and 4.6, respectively. Based on these results, not only are colonies of Formosan termites larger in nature and cause more damage to wooden structures than *R. flavipes*, but they are also more mobile.
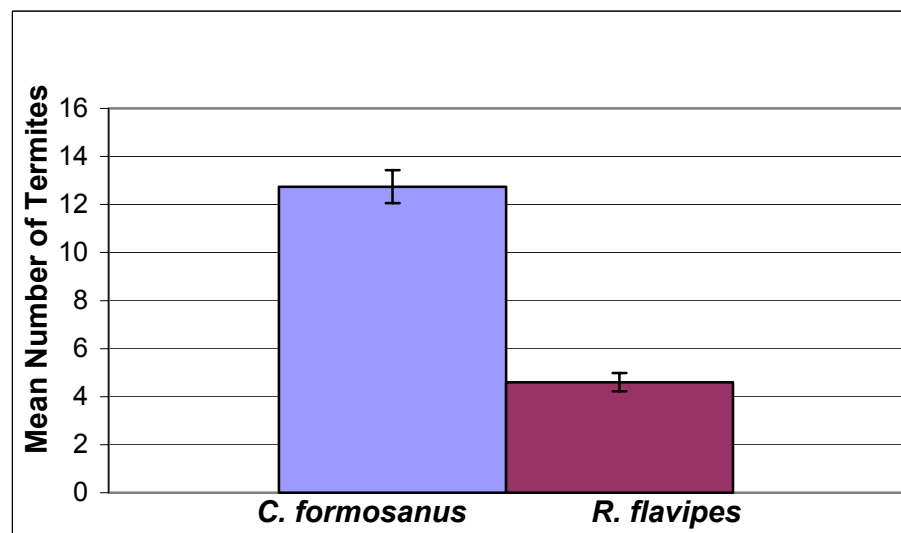
Figure 10. Summation of the mean number of *C. formosanus* and *R. flavipes* termites responding to the treatments in an olfactometer.

The results of the comparisons of *M. anisopliae* (C15) cultured on sterile corn and then aged for 1, 2, 4, 8, and 12 weeks, respectively before being tested in the olfactometer with three hundred workers of *R. flavipes* are shown in Figure 11. The most attractive treatment was C15 aged for 1 week prior to testing in the olfactometer. This treatment was significantly different ($p \leq 0.05$) than all the other treatments through time, with the exception of C15 aged for 2 weeks in the 2880 minute time trials where the results for the treatments were essentially the same. There appeared to be a trend for recruitment of *R. flavipes* with C15 aged for 1 and 2 weeks through time, while remaining treatments had consistently low levels of attractancy during testing. Initially the termite responses to the *M. anisopliae* treatments were relatively low; however, the untreated sterile corn control was apparently less attractive than treatments with the fungal mycelium, regardless of the age of mycelia prior to testing.
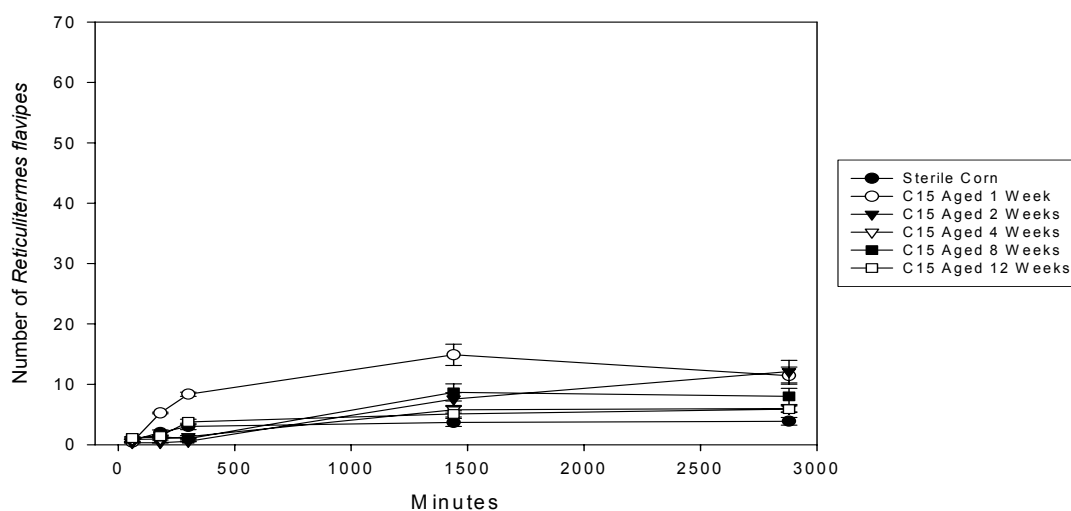
Figure 11. Mean number of *Reticulitermes flavipes* observed in the first generation of *M. anisopliae* cultured on corn (C15) or the control through time in an olfactometer.

The second generation of *M. anisopliae* (C16) cultured on sterile corn was tested in the olfactometer with 300 *R. flavipes*. The mycelium was aged for 1, 2, 4, 8, and 12 weeks prior to testing and the results are summarized in Figure 12. There were no significant differences between the untreated sterile corn control and C16 that had been aged 4, 8, and 12 weeks in the early stages of the experiment (60, 120, and 300 minutes). However, for all of the remaining test intervals, the non-treated control was less attractive than any of the C16 mycelia treatments. In these trials, *M. anisopliae* (C16) aged for only 2 weeks prior to testing was significantly (p≤0.05) more attractive than the other treatments through 2880 hours post-testing. It was also apparent from these trials that all treatments with C16 retained a measurable level of attractiveness through 12 weeks. The C16 treatments were consistently more attractive than the C15 treatments *R. flavipes* in these tests and noted in a comparison of Figures 11 and 12.
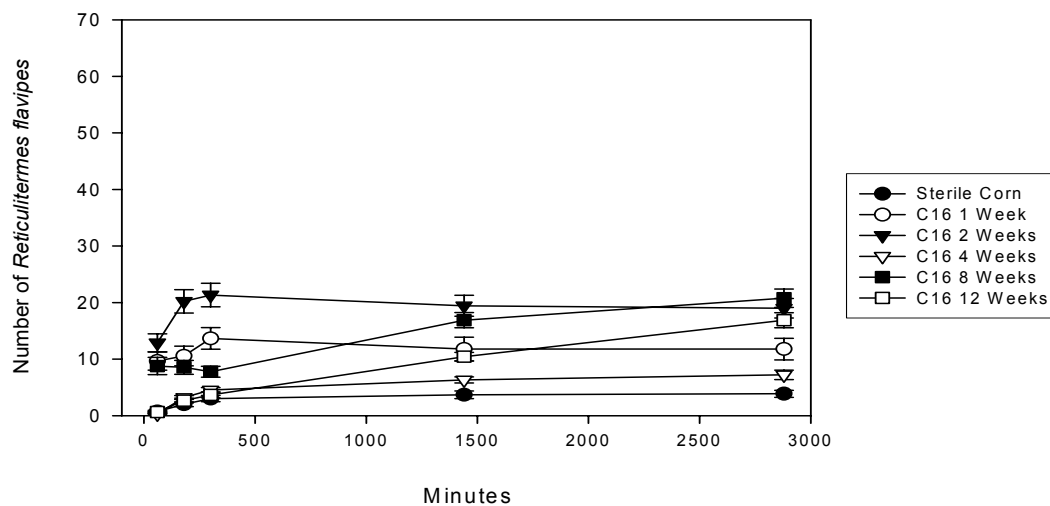
Figure 12. Mean number of *Reticulitermes flavipes* observed in the second generation of *M. anisopliae* cultured on corn (C16) or the control through time in an olfactometer.

When *M. anisopliae* was cultured on sterile rice (FF1000) and tested with 300 *R. flavipes* in an olfactometer, there were no significant differences in attractiveness during the first three test periods of 60, 120 and 300 minutes, with the exception of FF1000 that had been aged for 12 weeks. This treatment was significantly different ($p \leq 0.05$) than all of the other treatments through time and showed some evidence of recruitment of the other termites.

Also, at 1440 minutes, FF1000 aged for 1 and 2 weeks and the sterile rice control were significantly different ($p \leq 0.05$) from FF1000 aged for 4 and 8 weeks. At 2880 minutes, the treatment of FF1000 aged for 2 weeks was significantly different ($p \leq 0.05$) from FF1000 aged for 1, 4, and 8 weeks and the sterile rice control. The treatment of FF1000 aged for 1 week was significantly different ($p \leq 0.05$) from FF1000 aged for 4 and 8 weeks and the sterile rice control. The sterile rice control was significantly different ($p \leq 0.05$) from FF1000 aged for 4 and 8 weeks.

Another difference was that two of the treatments (FF1000 aged 4 and 8 weeks, respectively) were significantly less attractive than the untreated controls. The mean number of *R. flavipes* observed at each time period for the different treatments are shown in Figure 13.

Figure 13. Mean number of *Reticulitermes flavipes* observed in the first generation of *M. anisopliae* cultured on rice (FF1000) or the control through time in an olfactometer.

When olfactometer tests were conducted with 300 *C. formosanus* workers using *M. anisopliae* cultured on sterile corn (C15), at least two treatments were significantly different from the other two treatments. These were the untreated control of sterile corn and C15 aged for 2 weeks prior to testing (Figure 14). Mycelia aged for 1, 4, 8, and 12 weeks were approximately equal in attractiveness; however, these treatments were more attractive to *C. formosanus* (Figure 14) than to *R flavipes* (Figure 11). There was limited recruitment of termites to treatment chambers in the olfactometer after 300 minutes adjustment period.
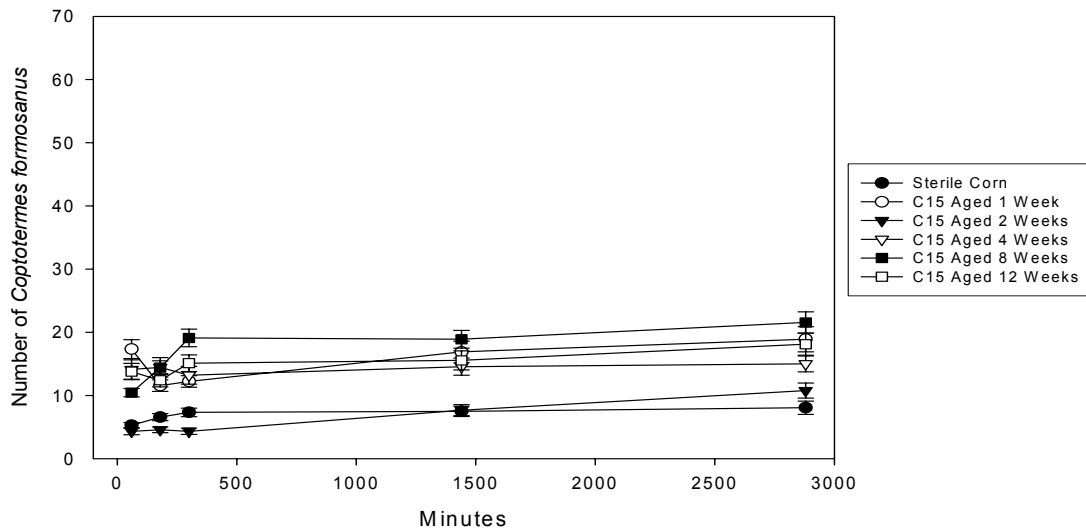
Figure 14. Mean number of *Coptotermes formosanus* observed in the first generation of *M. anisopliae* cultured on corn (C15) or the corn control through time in an olfactometer.

The second generation of *M. anisopliae* cultured on sterile corn (C16) and tested with *C. formosanus* was significantly (p≤0.05) more attractive than the untreated control (Figure 15). The most attractive cultures were those aged 4 and 12 weeks prior to testing in the olfactometer. Both of these treatments were significantly (p≤0.05) more attractive than either the sterile corn control or the other aged (C16) mycelia tested. There was a general trend for *C. formosanus* (Figure 15) to be more attracted to C16 than were *R. flavipes* (Figure 12).
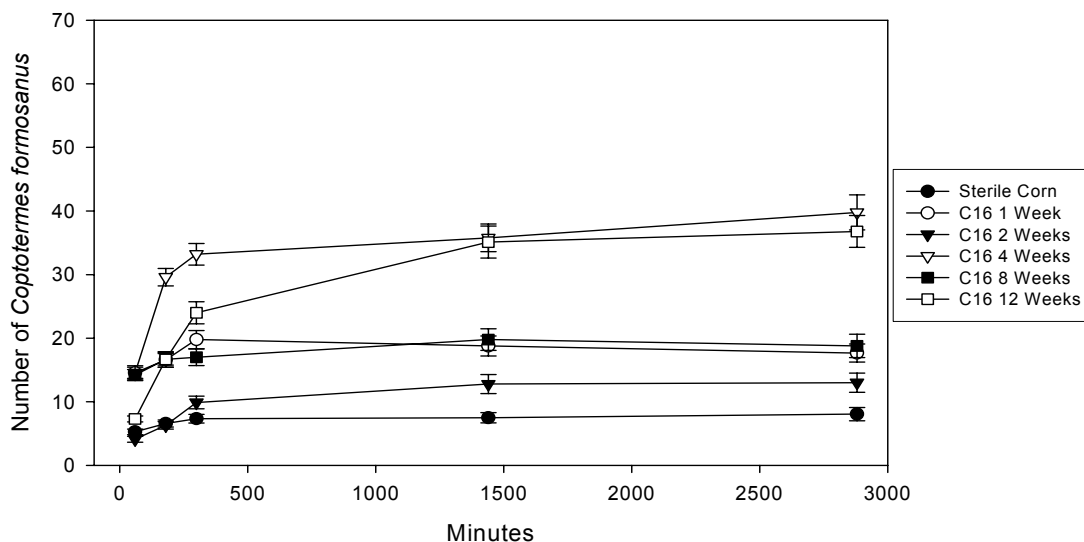
Figure 15. Mean number of *Coptotermes formosanus* observed in the second generation of *M. anisopliae* cultured on corn (C16) or the corn control through time in an olfactometer.

When *M. anisopliae* was cultured on sterile rice (FF1000) and tested in the olfactometer with 300 *C. formosanus,* there were significant ($p \leq 0.05$) preferences for the mycelia that had been aged for 8 weeks prior to testing. In all cases, after the first 300 minutes, all of the mycelia treatments were significantly different ($p \leq 0.05$) than the untreated sterile rice controls.

At 60 minutes, FF1000 aged for 4 and 8 weeks were significantly different ($p \leq 0.05$) from FF1000 aged for 1, 2, and 12 weeks and the sterile rice control. The FF1000 aged for 1 and 12 weeks and the sterile rice control were significantly different ($p \leq 0.05$) from FF1000 aged for 2 weeks.

At 180 minutes, FF1000 aged for 8 weeks was significantly different (p≤0.05) from FF1000 aged for 1, 2, 4, and 12 weeks and the sterile rice control. The FF1000 aged for 4 weeks was significantly different (p≤0.05) from FF1000 aged for 1, 2, and 12 weeks and the sterile rice control. The FF1000 aged for 1 and 12 weeks and the sterile rice control were significantly different (p≤0.05) from FF1000 aged for 2 weeks.

At 300 minutes, FF1000 aged for 8 weeks was significantly different (p≤0.05) from FF1000 aged for 1, 2, 4, and 12 weeks and the sterile rice control. The FF1000 aged for 4 weeks was significantly different (p≤0.05) from FF1000 aged for 1, 2, and 12 weeks and the sterile rice control. The FF1000 aged for 1 and 12 weeks and the sterile rice control were significantly different (p≤0.05) from FF1000 aged for 2 weeks.

At 1440 minutes, FF1000 aged for 8 weeks was significantly different (p≤0.05) from FF1000 aged for 1, 2, 4, and 12 weeks and the sterile rice control. The FF1000 aged for 2 and 4 weeks were significantly different (p≤0.05) from FF1000 aged for 1 and 12 weeks and the sterile rice control. The FF1000 aged for 1 and 12 weeks differed significantly (p≤0.05) from the sterile rice control.

At 2880 minutes, FF1000 aged for 8 weeks was significantly different (p≤0.05) from FF1000 aged for 1, 2, 4, and 12 weeks and the sterile rice control. The FF1000 aged for 2 weeks was significantly different (p≤0.05) from FF1000 aged for 1, 4, and 12 weeks and the sterile rice control. The FF1000 aged for 4 weeks was significantly different (p≤0.05) from FF1000 aged for 1 and 12 weeks and the sterile rice control. The FF1000 aged for 1 and 12 weeks were significantly different (p≤0.05) from the sterile rice control. After the initial adjustment period, the first generation of mycelium cultured on

rice (FF1000) was significantly (p≤ 0.05) more attractive than the sterile rice control at 1440 minutes. The mean number of *C. formosanus* observed at each time period for the different treatments are shown in Figure 16.
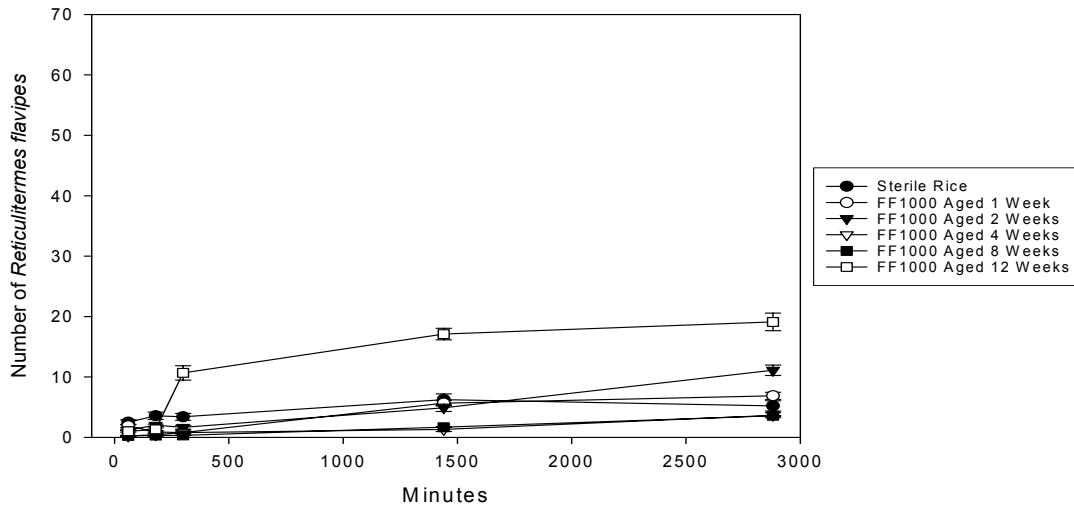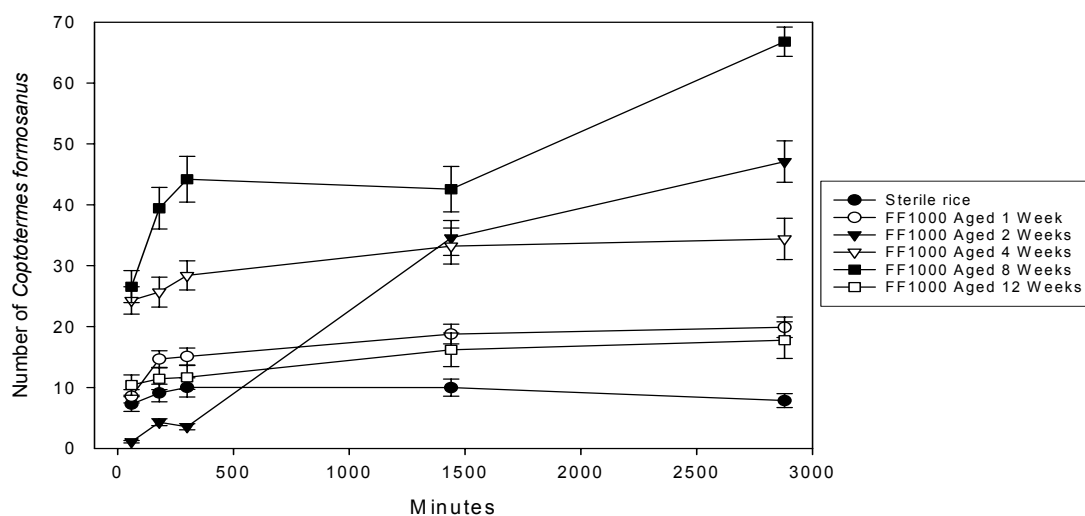

Figure 16. Mean number of *Coptotermes formosanus* observed in the first generation of *M. anisopliae* cultured on rice (FF1000) or the rice control through time in an olfactometer.

Ethanol (40%) extracts were prepared from *M. anisopliae* mycelium cultured on sterile rice (X-5). Comparisons were made in an olfactometer with 300 *R. flavipes*. The comparisons included dilutions of X-5 at 1:0, 1:10, 1:100, and 1:1000, an ethanol control, and Summon®. The results of these comparisons are in Figure 17. The most preferred treatments were the 1:1000 dilution of X-5 and Summon®, both of which were significantly different (p≤0.05) from each other as well as from the other treatments

(Figure 17). The least preferred treatment was the 1:10 dilution of X-5 extract, which

was significantly different (p≤0.05) from all other treatments. There were no significant

differences between the 1:0 dilution of X-5, the ethanol control, and the 1:100 dilution of

X-5 extract. Of the six treatments, the only one that showed potential recruitment by

foraging workers of *R. flavipes* was the 1:1000 dilution of X-5 extract (Figure 17).

There was a consistent increase in response by the termites to this dilution of the mycelia

extract through time.
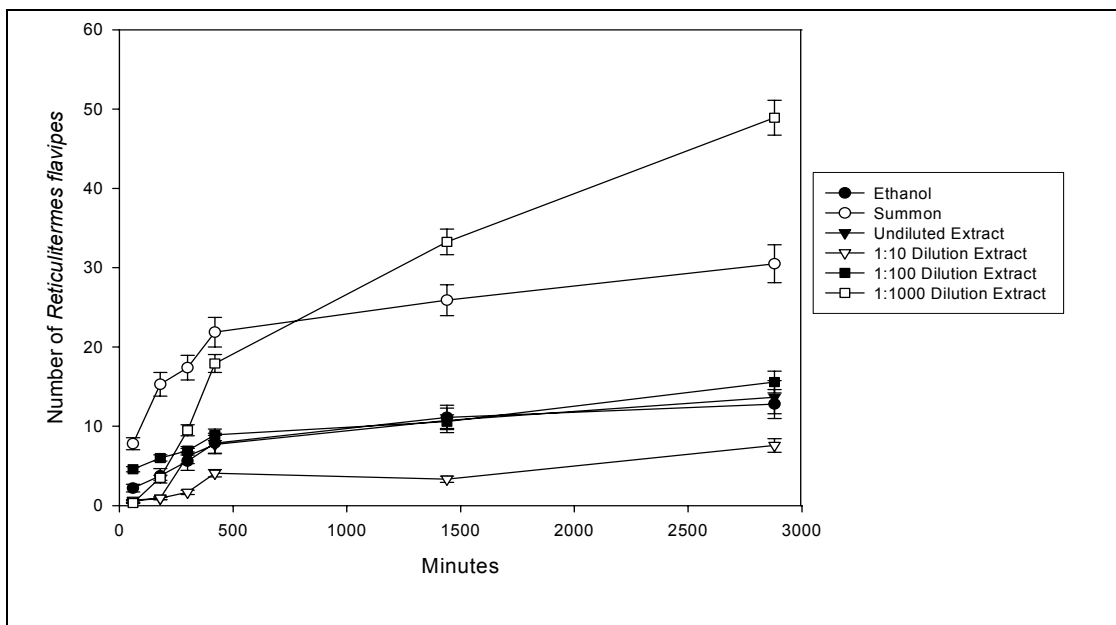
 Figure 17. The mean number of *Reticulitermes flavipes* found in each treatment when
testing the ethanol extract of *M. anisopliae* (X-5) cultured on rice and Summon® disk
through time in an olfactometer.

Glass Tube Bioassays: A summary of the tunneling distances in all of the bioassay tubes conducted with *C. formosanus* and *R. flavipes* workers is presented in Figure 18. The overall mean tunneling distances for *C. formosanus* and *R. flavipes* were 66.5 and 61.1 mm, respectively. *Coptotermes formosanus* tunneled significantly ($p \leq 0.05$) farther than *R. flavipes* in these bioassay tubes. An evaluation of the individual trials with various treatments is presented in Figures 18-21.
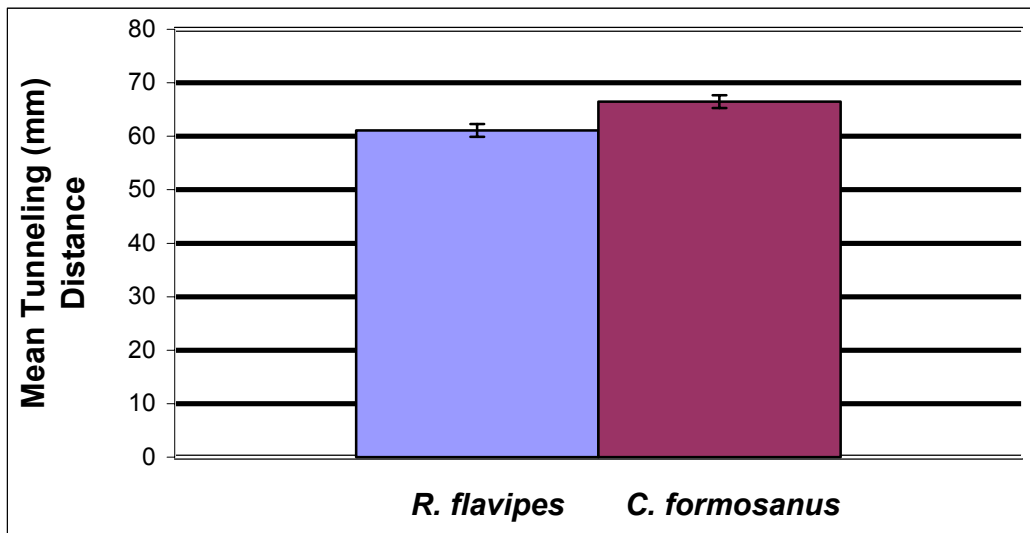
Figure 18. The mean tunneling distances for all treatments for *R. flavipes* and *C. formosanus* in the bioassay tubes held for 6 days at 25° C.

When 30 *R. flavipes* were provided access to aged mycelium of *M. anisopliae* cultured on sterile corn (C15), there were no significant differences between treatments (Figure 19). The termite workers were able to penetrate the bioassay tubes within the 6

day test period. Whereas there was less tunneling in the untreated soil and sterile corn

controls, these differences were not significant. The presence of mycelium in the

treatments apparently did not inhibit tunneling in these tests.
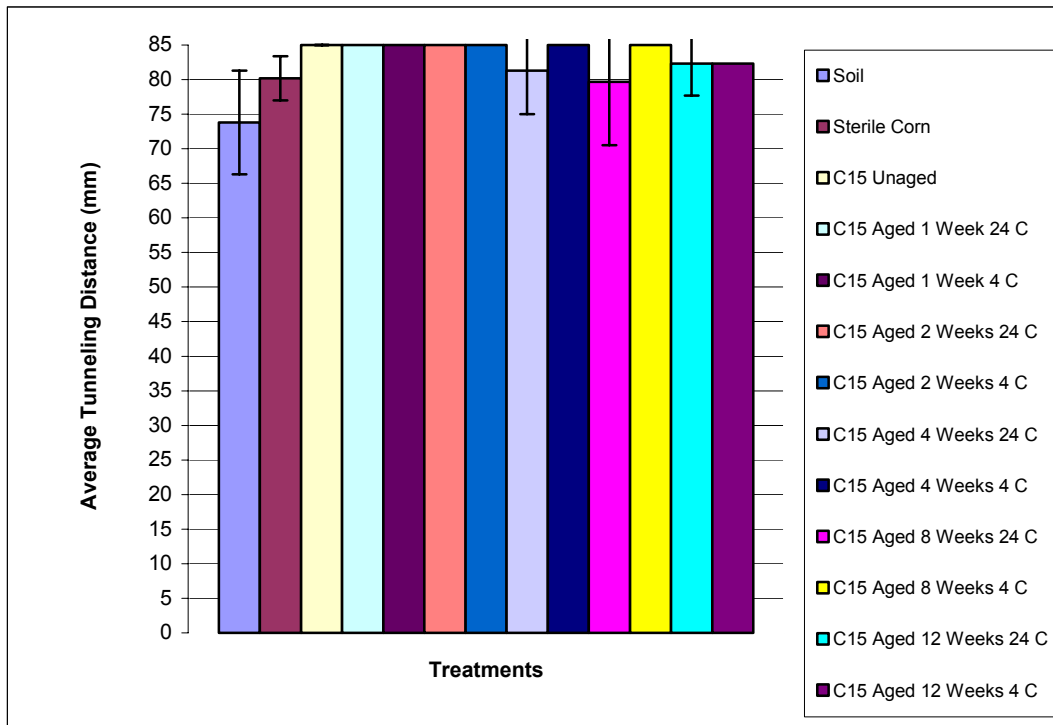
Figure 19. The mean tunneling distance of thirty *R. flavipes* when exposed to two temperatures and five different ages of the first generation *M. anisopliae* cultured on corn (C15) for 6 days in bioassay tubes held at 25° C.

In the bioassay tubes that included the second generation of *M. anisopliae* cultured on

corn (C16) and *R. flavipes*, there was a disruption of tunneling with the mycelium that

had been aged for 2 weeks at 4º C and C16 aged for 12 weeks at 24º C (Figure 20).

These treatments were not significantly different ($p \leq 0.05$) from each other or from the

untreated controls of soil and sterile corn, but they were significantly different than other

C16 mycelia treatments. The presence of C16 mycelium was not a consistent tunneling inhibitor in these tests.
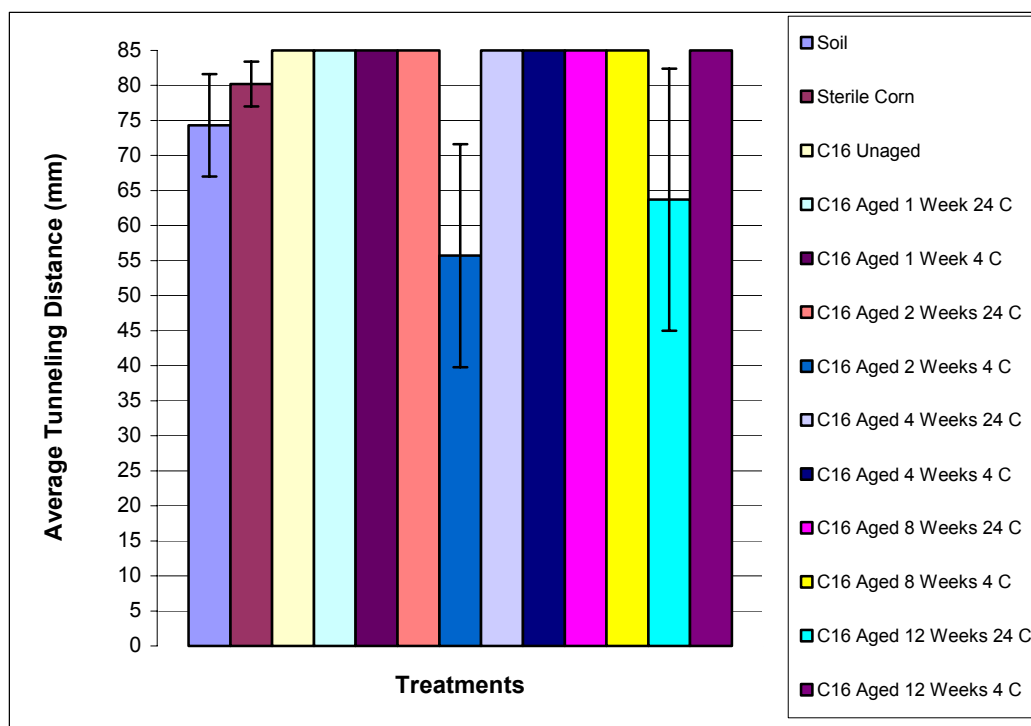
Figure 20. The mean tunneling distance of thirty *R. flavipes* when exposed to two temperatures and five different ages of the second generation of *M. anisopliae* cultured on corn (C16) for 6 days in bioassay tubes held at 25° C.

When *M. anisopliae* was cultured on sterile rice (FF1000) and tested with thirty *R. flavipes* in the bioassay tubes, there was a strong correlation between the aged mycelium and inhibition of tunneling (Figure 21). The termites entered the portion of the bioassay tubes that contained mycelium and spent up to 6 days in that area. The termites were able to tunnel completely through the non-treated sterile rice controls, which indicated that it was the mycelium that was inhibiting tunneling. In general, there were significant

differences between the treatments with aged FF1000, compared to the untreated

controls.
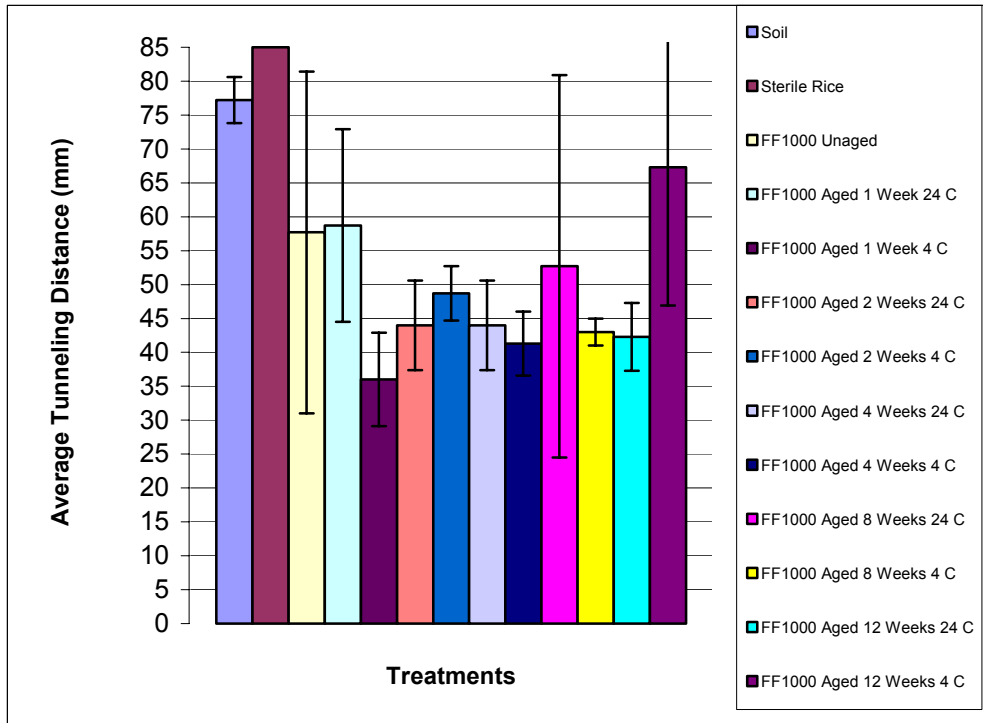
Figure 21. The mean tunneling distance of thirty *R. flavipes* when exposed to two
temperatures and five different ages of the first generation of *M. anisopliae* cultured on
rice (FF1000) for 6 days in bioassay tubes held at 25° C.

The results of bioassay tube experiments conducted on thirty workers of *C.
formosanus* allowed access to mycelia of *M. anisopliae* are found in Figures 22-24.

When the first generation of *M. anisopliae* was cultured on sterile corn (C15) and aged

from 1 to 12 weeks at 24 or 4º C, there were no significant differences between the soil

and sterile corn controls and aged mycelium (Figure 22). Apparently, mycelium did not inhibit tunneling by *C. formosanus* in these tests.

When the second generation of *M. anisopliae* was cultured on sterile corn (C16) and exposed to foraging *C. formosanus* in bioassay tubes, there were no significant differences between the treatments (Figure 23).
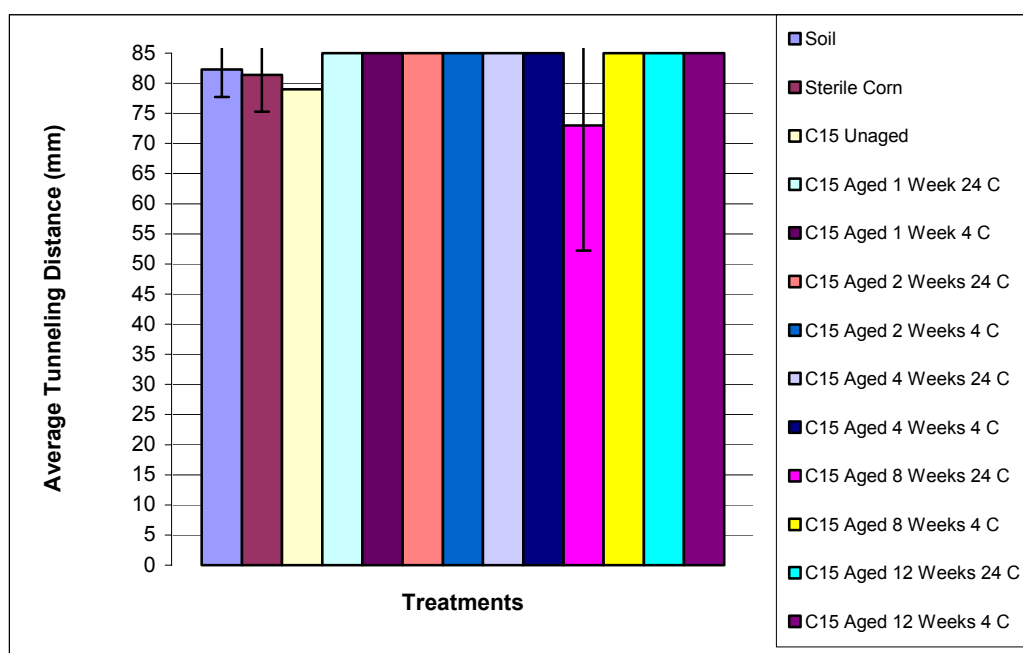
Figure 22. The mean tunneling distance of thirty *C. formosanus* when exposed to two temperatures, and five different ages of the first generation of *M. anisopliae* cultured on corn (C15) for 6 days in bioassay tubes held at 25° C.
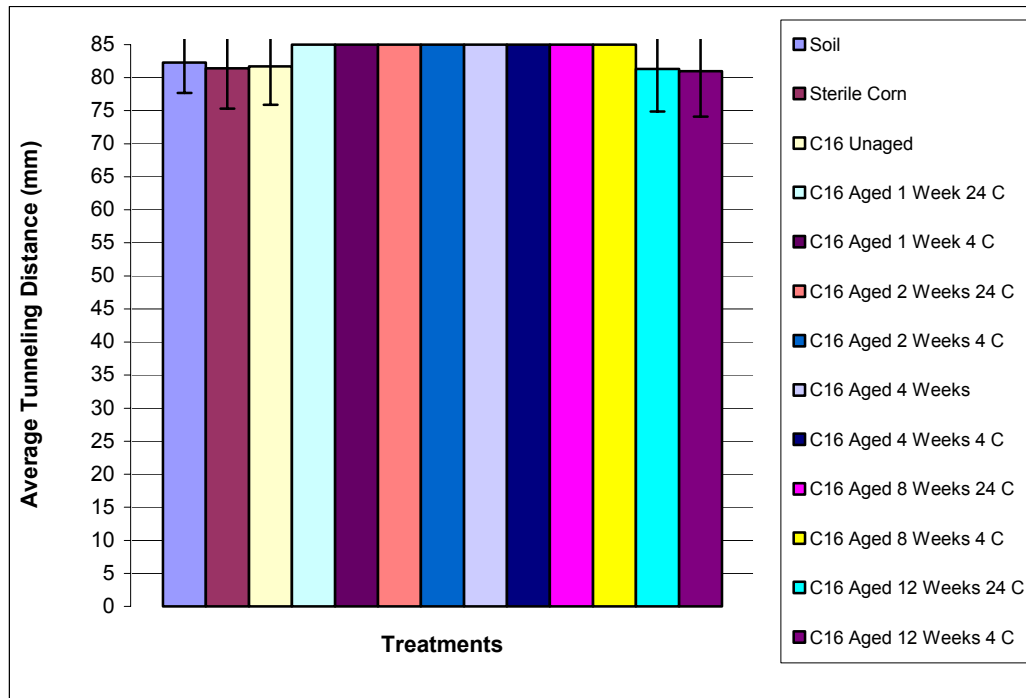
Figure 23. The mean tunneling distance of thirty *C. formosanus* when exposed to two temperatures and five different ages of the second generation of *M. anisopliae* cultured on corn (C16) for 6 days in bioassay tubes held at 25° C.

When *M. anisopliae* was cultured on sterile rice (FF1000) and provide 30 *C. formosanus* workers in bioassay tubes, there were significant differences (p≤0.05) between all of the FF1000 treatments (Figure 24), except FF1000 aged for 1 week at 24º C, and the soil and sterile rice controls. It was apparent that foraging termites tunneled to the area containing mycelium, and remained there for the 6 day testing period. These effects were due to the presence of aged mycelium and not to sterile rice. Mycelium effectively inhibited or attracted *C. formosanus,* even when it was aged for 12 weeks at either 4 or 24º C. These results are similar to those determined for *R. flavipes* exposed to FF1000 (Figure 21).
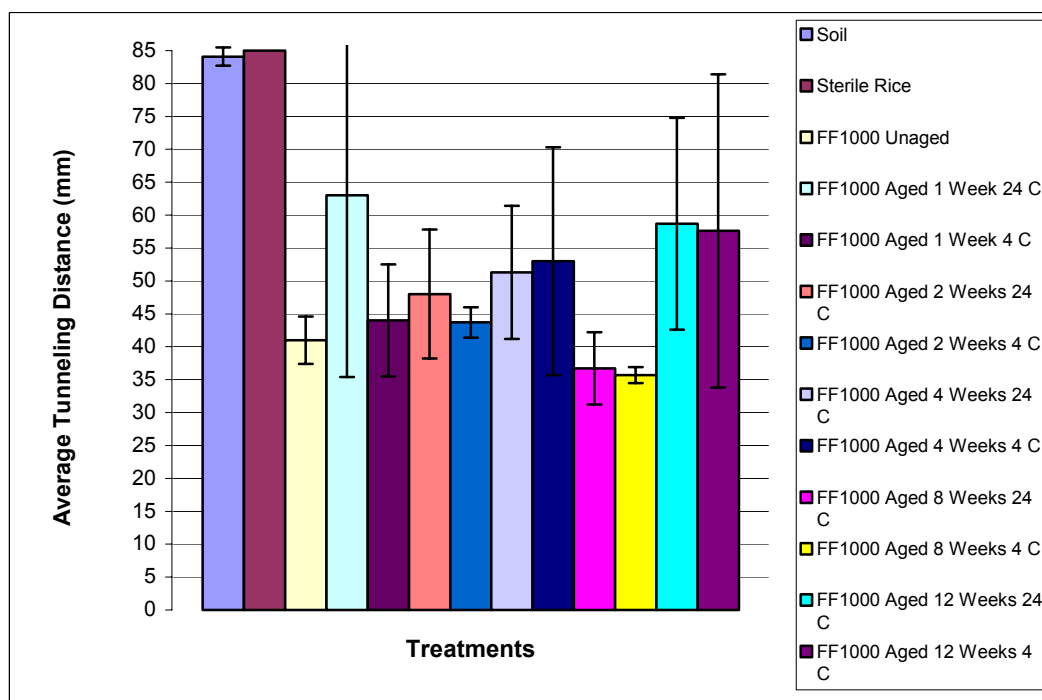
Figure 24. The mean tunneling distance of thirty *C. formosanus* when exposed to two temperatures and five different ages of the first generation of *M. anisopliae* cultured on rice (FF1000) for 6 days in bioassay tubes held at 25° C.

At the end of 6 days, the bioassay tubes were carefully disassembled and live termites were counted. Mortality rates were then determined for each treatment, and the results are provided in Tables 1-6.

For *R. flavipes*, the mortality found in the bioassay tubes containing the unaged treatment of the first generation of *M. anisopliae* cultured on corn (C15) was significantly different (p≤0.05) from the soil and sterile corn controls. Aged C15 for 1 week was significantly different (p≤0.05) from C15 aged for 1 week at 4º C, soil and sterile corn controls. Aged C15 for 2 weeks at 24 and 4º C were significantly different (p≤0.05) from the soil and sterile corn controls. Aged C15 for 4 weeks at 24 and 4º C

were significantly different ($p \leq 0.05$) from the soil control, and the soil control was

significantly different ($p \leq 0.05$) from the sterile corn control. Aged C15 for 12 weeks at

24 and 4º were significantly different ($p \leq 0.05$) from the soil and sterile corn controls.

The mortality found in the mycelia treatments at the end of 6 days in this experiment

were significantly different ($p \leq 0.05$) compared to the soil controls.

The lowest mortality was observed in the soil and sterile corn treatments. The first

generation of mycelium cultured on corn (C15) that was aged for 12 weeks produced the

highest mortality both at 4° C and 24° C, and were significantly different ($p \leq 0.05$) from

the soil and sterile corn controls. The mean percent mortality and the standard deviation

observed in each treatment are shown in Table 1.

Table 1. The mean percent mortality and the standard deviation of thirty
R. flavipes when exposed to the first generation of *M. anisopliae* cultured on corn (C15)
at various different temperatures and ages for 6 days in bioassay tubes.

Treatment	Exposed Temperature	Unaged[2]	1 Week[2]	2 Weeks[2]	4 Weeks[2]	8 Weeks[2]	12 Weeks[2]
First generation of *M. anisopliae* cultured on corn	24° C	11.0 ± 1.5^a	11.0 ± 1.3^a	15.6 ± 2.1^a	6.7 ± 2.0^a	10.0 ± 2.7^a	23.4 ± 2.1^a
First generation of *M. anisopliae* cultured on corn	4° C	[1]	1.0 ± 1.0^b	3.3 ± 1.0^a	7.7 ± 0.6^a	4.3 ± 2.3^a	20.0 ± 2.6^a
Soil	24° C	1.1 ± 0.6^b	0.0 ± 0.0^b	1.1 ± 0.6^b	0.0 ± 0.0^b	1.1 ± 0.6^a	0.0 ± 0.0^b
Sterile Corn	4° C	2.2 ± 0.6^b	1.1 ± 0.6^b	1.1 ± 0.6^b	2.2 ± 0.6^a	0.0 ± 0.0^a	3.3 ± 0.0^b

[1]Could not be tested
[2] Means followed by the same letter within the same column were not significantly
different ($p \geq 0.05$) from each other using Univariate Analysis of Variance (SPSS, 2001).

For *R. flavipes*, mortality associated with unaged C16 was significantly different (p≤0.05) from soil and sterile corn controls. Aged C16 for 2 weeks at 4º C was significantly different (p≤0.05) from C16 aged for 2 weeks at 24º C, soil and sterile corn controls. Mortality for C16 aged for 4 weeks at 24 and 4º C was significantly different (p≤0.05) from soil and sterile corn controls. Aged C16 for 8 weeks at 24º C was significantly different (p≤0.05) from C16 aged for 8 weeks at 4º C, soil and sterile corn controls. Aged C16 for 8 weeks at 4º C was significantly different (p≤0.05) from soil and sterile corn controls. Mortality for C16 aged for 12 weeks at 24 and 4º C were significantly different (p≤0.05) from the soil and sterile corn controls.

The lowest mortality was observed in soil and sterile corn treatments. The second generation of mycelium cultured on corn (C16) that was aged for 12 weeks showed the highest mortality both at 4° C and 24° C, and were significantly different (p≤ 0.05) from the soil and sterile corn controls. The mean percent mortality and the standard deviation observed in each treatment are shown in Table 2.

Table 2. The mean percent mortality and the standard deviation of thirty
R. flavipes when exposed to the second generation of *M. anisopliae* cultured on corn
(C16) at two different temperatures and 6 ages for 6 days in bioassay tubes.

Treatment	Exposed Temperature	Unaged[2]	1 Week[2]	2 Weeks[2]	4 Weeks[2]	8 Weeks[2]	12 Weeks[2]
Second generation of *M. anisopliae* cultured on corn	24° C	11.0 ± 3.5^a	1.0 ± 0.6^a	3.3 ± 1.7^a	15.6 ± 3.5^a	24.3 ± 1.2^a	36.7 ± 2.6^a
Second generation of *M. anisopliae* cultured on corn	4° C	[1]	2.2 ± 1.2^a	20.0 ± 2.6^b	8.9 ± 2.1^a	12.7 ± 2.3^b	33.3 ± 3.6^a
Soil	24° C	1.1 ± 0.6^b	0.0 ± 0.0^a	1.1 ± 0.6^a	0.0 ± 0.0^b	1.1 ± 0.6^c	0.0 ± 0.0^b
Sterile Corn	4° C	2.2 ± 0.6^b	0.0 ± 0.0^a	1.1 ± 0.6^a	2.2 ± 0.6^b	0.0 ± 0.0^c	3.3 ± 0.0^b

[1]Could not be tested

[2] Means followed by the same letter within the same column were not significantly
different ($p \geq 0.05$) from each other using Univariate Analysis of Variance (SPSS, 2001).

For *R. flavipes,* mortality associated with FF1000 aged for 1 week at 24 and 4º C
were significantly different ($p \leq 0.05$) from that for soil and sterile rice controls. Aged
FF1000 for 8 weeks at 4º C was significantly different ($p \leq 0.05$) from FF1000 aged for 8
weeks at 24º C, and soil and sterile rice controls.

The lowest mortality was observed in the soil and sterile rice treatments. The first
generation of mycelium cultured on rice (FF1000) that was aged for 1 week showed the
highest mortality both at 4 and 24° C and these mortalities were significantly different
($p \leq 0.05$) from these for soil and sterile rice controls. The mortality effects of exposure
of *R. flavipes* to *M. anisopliae* were maintained for up to 12 weeks. The mean percent
mortality and the standard deviation observed in each treatment are shown in Table 3.

Table 3. The mean percent mortality and the standard deviation of thirty
R. flavipes when exposed to the first generation of *M. anisopliae* cultured on rice
(FF1000) at two different temperatures and aged for 6 days in bioassay tubes.

Treatment	Exposed Temperature	Unaged[2]	1 Week[2]	2 Weeks[2]	4 Weeks[2]	8 Weeks[2]	12 Weeks[2]
First generation of *M. anisopliae* cultured on rice	24° C	2.2 ± 0.6^a	36.7 ± 4.0^a	11.0 ± 0.6^a	4.3 ± 1.5^a	6.7 ± 2.9^a	11.0 ± 2.1^a
First generation of *M. anisopliae* cultured on rice	4° C	[1]	31.0 ± 2.5^a	7.7 ± 0.6^a	9.2 ± 1.3^a	13.3 ± 1.8^b	15.9 ± 2.4^a
Soil	24° C	0.0 ± 0.0^a	1.1 ± 0.6^b	1.1 ± 0.6^a	1.1 ± 0.6^a	0.0 ± 0.0^a	0.0 ± 0.0^b
Sterile Rice	4° C	2.2 ± 0.6^a	1.3 ± 0.6^b	1.3 ± 0.6^a	1.1 ± 0.6^a	3.3 ± 0.0^a	2.2 ± 0.6^b

[1]Could not be tested

[2] Means followed by the same letter within the same column were not significantly
different ($p \geq 0.05$) from each other using Univariate Analysis of Variance (SPSS, 2001).

The most significant effects of *M. anisopliae* aging were observed in the 8 and 12
week trials (Table 3). For *C. formosanus,* C15 aged for 2 weeks at 24° C was
significantly different ($p \leq 0.05$) from C15 aged for 2 weeks at 4° C, soil and sterile corn
controls. Aged C15 for 4 weeks at 24 and 4° C were significantly different ($p \leq 0.05$)
from the soil and sterile corn controls. Aged C15 for 8 weeks at 24° C was significantly
different ($p \leq 0.05$) from C15 aged for 8 weeks at 4° C, soil and sterile corn controls, and
C15 aged for 8 weeks at 4° C was significantly different ($p \leq 0.05$) from soil and sterile
corn controls. The C15 aged for 12 weeks at 24 and 4° C were significantly different
($p \leq 0.05$) from soil and sterile corn controls.

The lowest mortality was observed in soil and sterile corn treatments. The first
generation of mycelium cultured on corn (C15) and aged for 8 weeks showed the highest

mortality at 24° C, and this mortality was significantly different (p≤0.05) from that for

mycelia aged for 8 weeks at 4° C, and the soil and sterile corn controls. The mycelium

aged for 12 weeks at 4° C showed the highest mortality. The mycelium aged for 12

weeks at 4° C was significantly different (p≤ 0.05) from the soil and sterile corn

controls. The mean mortality and standard deviation observed in each treatment for *C.*

formosanus are shown in Table 4.

Table 4. The mean percent mortality and the standard deviation of thirty
C. formosanus when exposed to the first generation of *M. anisopliae* cultured on corn
(C15) at two different temperatures and 6 ages for 6 days in bioassay tubes.

Treatment	Exposed Temperature	Unaged[2]	1 Week[2]	2 Weeks[2]	4 Weeks[2]	8 Weeks[2]	12 Weeks[2]
First generation of *M. anisopliae* cultured on corn	24° C	7.7 ± 2.5^a	5.7 ± 1.5^a	16.7 ± 3.6^a	14.3 ± 2.3^a	31.0 ± 7.1^a	20.0 ± 5.0^a
First generation of *M. anisopliae* cultured on corn	4° C	[1]	4.3 ± 1.5^a	3.3 ± 1.0^b	9.2 ± 0.6^a	13.3 ± 3.8^b	15.9 ± 1.2^a
Soil	24° C	1.1 ± 0.6^a	0.0 ± 0.0^a	1.1 ± 0.6^b	3.3 ± 0.0^b	3.3 ± 0.6^c	0.0 ± 0.0^b
Sterile Corn	4° C	2.2 ± 0.6^a	1.1 ± 0.6^a	0.0 ± 0.0^b	1.1 ± 0.6^b	1.1 ± 0.6^c	0.0 ± 0.0^b

[1]Could not be tested
[2] Means followed by the same letter within the same column were not significantly
different (p≥ 0.05) from each other using Univariate Analysis of Variance (SPSS, 2001).

The results of testing with aged mycelium of *M. anisopliae* on the mortality of *C.*

formosanus is presented in Table 5. For *C. formosanus,* C16 aged for 4 weeks at 24º C

was significantly different (p≤0.05) from C16 aged for 4 weeks at 4º C, soil and sterile

corn controls. Aged C16 for 12 weeks at 24 and 4º C were significantly different

(p≤0.05) from the soil and sterile corn controls.

The lowest mortality was observed in the soil and sterile corn treatments. The second

generation of mycelium cultured on corn (C16) and aged for 4 weeks showed the highest

mortality at 24° C, and the mortality was significantly different (p≤0.05) from that for

mycelia aged for 4 weeks at 4° C, and the soil and sterile corn controls. The mycelium

aged for 12 weeks at 4° C showed the highest mortality and was significantly different

(p≤ 0.05) from the soil and sterile corn controls. The mean mortality and standard

deviation observed in each treatment for *C. formosanus* are shown in Table 5.

Table 5. The mean percent mortality and the standard deviation of thirty
C. formosanus when exposed to the second generation of *M. anisopliae* cultured on corn
(C16) at two different temperatures and 6 ages for 6 days in bioassay tubes.

Treatment	Exposed Temperature	Unaged[2]	1 Week[2]	2 Weeks[2]	4 Weeks[2]	8 Weeks[2]	12 Weeks[2]
Second generation of *M. anisopliae* cultured on corn	24° C	5.3 ± 1.5^a	3.5 ± 1.3^a	4.3 ± 1.5^a	11.0 ± 1.5^a	2.2 ± 1.2^a	8.9 ± 2.1^a
Second generation of *M. anisopliae* cultured on corn	4° C	[1]	4.3 ± 0.6^a	2.2 ± 1.2^a	1.7 ± 1.8^b	1.0 ± 0.6^a	14.3 ± 1.2^a
Soil	24° C	0.0 ± 0.0^a	0.0 ± 0.0^a	1.1 ± 0.6^a	3.3 ± 0.0^b	3.3 ± 0.0^a	0.0 ± 0.0^b
Sterile Corn	4° C	0.0 ± 0.0^a	1.1 ± 0.6^a	0.0 ± 0.0^a	1.1 ± 0.6^b	1.1 ± 0.6^a	0.0 ± 0.0^b

[1]Could not be tested
[2]Means followed by the same letter within the same column were not significantly
different (p≥ 0.05) from each other using Univariate Analysis of Variance (SPSS, 2001).

The results of testing with aged mycelium of *M. anisopliae* cultured on rice (FF1000) on the mortality of *C. formosanus* are summarized in Table 6. For *C. formosanus,* mortality associated with FF1000 aged for 1 week at 24 and 4º C was significantly different from the soil and sterile rice controls. The FF1000 aged for 2 weeks at 24º C was significantly different from FF1000 aged for 2 weeks at 4º C, soil and sterile corn controls. The FF1000 aged for 4 weeks at 24 and 4º C were significantly different from the soil and sterile rice controls. The FF1000 aged for 8 weeks at 24 and 4º C were significantly different from the soil and sterile rice controls. The FF1000 aged for 12 weeks at 24 and 4º C were significantly different from the soil and sterile corn controls.

The lowest mortality resulting from exposure to FF1000 was observed in the soil and sterile rice treatments. The first generation of mycelium cultured on rice (FF1000) that was aged for 1 week showed the highest mortality both at 4 and 24° C and were significantly different ($p \leq 0.05$) from the soil and sterile rice controls. There was a low but significant level of mortality, associated with mycelia aged from 1 to 12 weeks. The mean mortality and standard deviation observed in each treatment for *C. formosanus* are shown in Table 6. There was a significant difference ($p \leq 0.05$) in mortality in *C. formosanus* exposed to FF1000 cultured on rice rather than corn (C15 and C16).

Table 6. The mean percent mortality and the standard deviation of thirty
C. formosanus when exposed to the first generation of *M. anisopliae* cultured on rice
(FF1000) at two different temperatures and 6 ages for 6 days in bioassay tubes.

Treatment	Exposed Temperature	Unaged[2]	1 Week[2]	2 Weeks[2]	4 Weeks[2]	8 Weeks[2]	12 Weeks[2]
First generation of *M. anisopliae* cultured on rice	24° C	2.2 ± 1.2^a	21.0 ± 1.2^a	16.7 ± 3.0^a	15.5 ± 1.2^a	13.3 ± 2.6^a	15.6 ± 1.2^a
First generation of *M. anisopliae* cultured on rice	4° C	[1]	22.2 ± 1.5^a	5.6 ± 0.6^b	11.4 ± 1.2^a	13.3 ± 3.6^a	20.0 ± 2.6^a
Soil	24° C	0.0 ± 0.0^a	0.0 ± 0.0^b	0.0 ± 0.0^b	0.0 ± 0.0^b	1.1 ± 0.6^b	0.0 ± 0.0^b
Sterile Rice	4° C	2.2 ± 0.6^a	3.3 ± 0.0^b	2.2 ± 0.6^b	2.2 ± 0.6^b	2.2 ± 0.6^b	2.2 ± 0.6^b

[1]Could not be tested
[2] Means followed by the same letter within the same column were not significantly
different ($p \geq 0.05$) from each other using Univariate Analysis of Variance (SPSS, 2001).

Glass Plate Bioassays: The results of *R. flavipes* observed at each of the seven

treatments are shown in Figure 25. The results indicated that Summon®, untreated

disks, and ethanol treated disks, although acceptable to foraging *R. flavipes*, did not

recruit overtime. The number of termites visiting these disks did not significantly

increase over a 12 day period. There was evidence of recruitment by *R. flavipes* to all

dilutions of the *M. anisopliae* ethanol extract (X-5) cultured on sterile rice through time.

R. flavipes preferences, from the least to the greatest, occur in the following order:

Summon®, ethanol, untreated, 1:0 dilution of X-5 extract, 1:10 dilution of X-5 extract,

1:100 dilution of X-5 extract, and 1:1000 dilution of X-5 extract. Clearly, *M. anisopliae*

extract dilutions were preferred over other treatments; however, there was an inverse

relationship, in which more recruitment was observed for 1:1000 dilutions and the least

recruitment was with undiluted treatments. The 1:1000 dilution of *M. anisopliae* ethanol

extract (X-5) was significantly different (p≤ 0.05) from the other treatments throughout

the study. The mean number of termites observed at each treatment for each of the 12

days is shown in Figure 25.
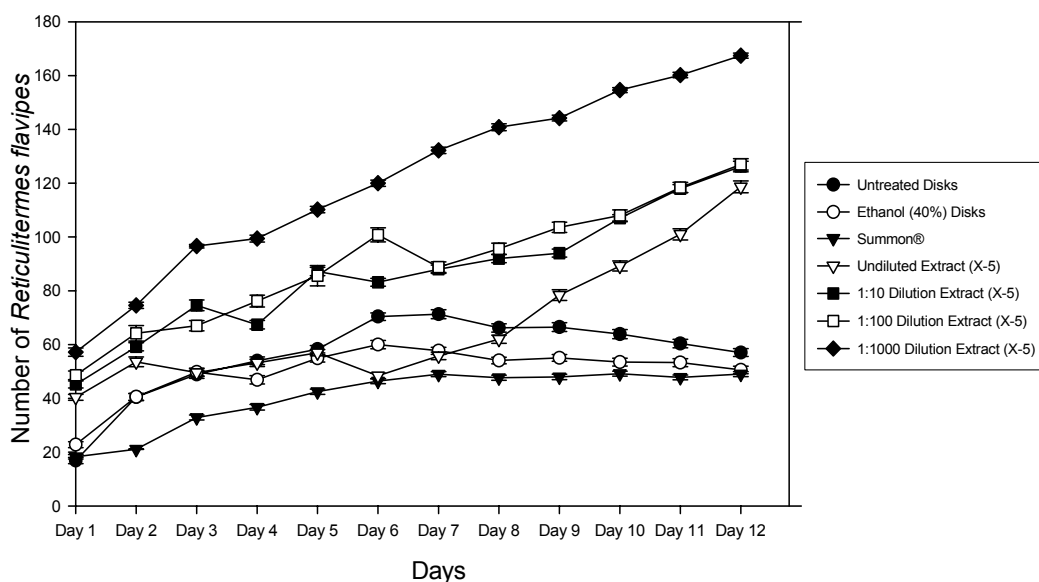
Figure 25. Mean number and standard error of *Reticulitermes flavipes* observed around each treatment for 12 days in the glass plate bioassays.

The results of the tests that compared consumption of the cellulose matrix associated

with each of the treatments are summarized in Figure 26. The largest difference between

the initial and the final weight of the treatment disks after 12 days was observed for

Summon® followed, in order of decreasing consumption levels, by 1:0 dilution of X-5

extract, 1:10 dilution of X-5 extract, 1:100 dilution of X-5 extract, ethanol treated disks,

and untreated disks, respectively. The smallest difference in weight was associated with

the 1:1000 dilution of *M. anisopliae* ethanol extract (X-5) treated disks. The initial

weight and final weight of each treatment is shown in Appendix A, and the amount

consumed by *R. flavipes* in each treatment is shown in Figure 26.
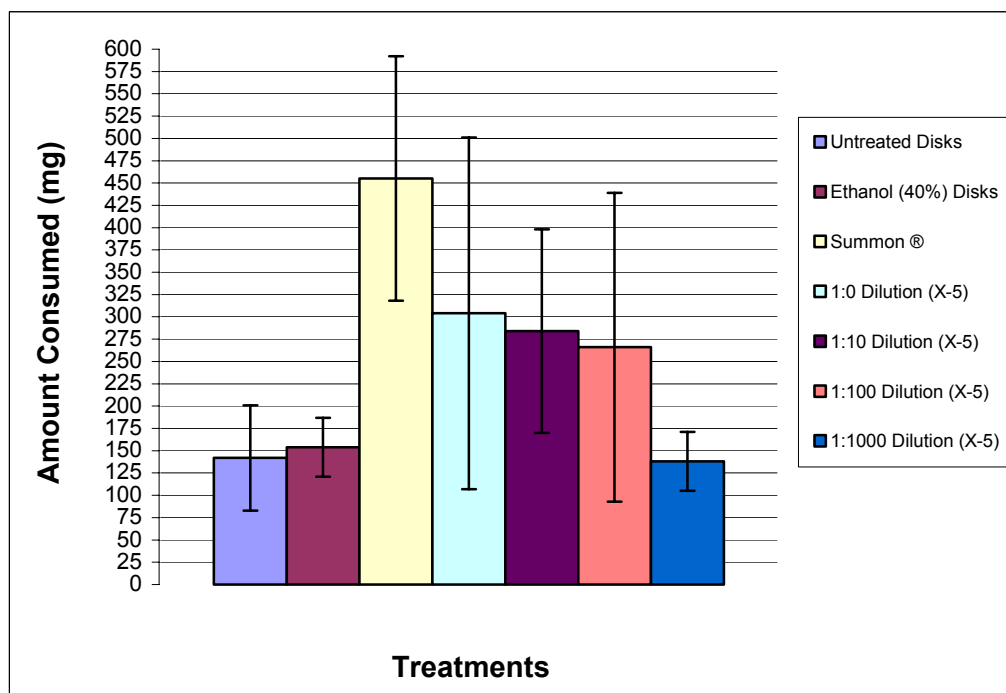
Figure 26. The amount (mg) of each treatment consumed by *Reticulitermes flavipes*
after 12 days in the glass plate bioassay study.

Field Evaluation: The monitoring stations around each of the five urban structures

were observed between 27 and 32 times, depending upon the date of installation and

weather conditions. For all urban structures, the highest numbers of visits by termites

were observed for *M. anisopliae* ethanol extract (X-5) treated monitors, which were

significantly different (p≤ 0.05) from other treated monitors for July, August, and

September. The lowest numbers of visits were observed for untreated monitors, and

these numbers were significantly different ($p \leq 0.05$) from those for other monitors and

identical time periods. There was a decline in termite activity in the Summon®,

undiluted extract, and ethanol control. Summon® preferred food source did not recruit

termites to the bait stations during the test interval. The mean numbers of visits

observed at each structure for each treatment at each time interval are shown in Figures

27-31. The undiluted extract of *M. anisopliae* was always preferred to other treatments.
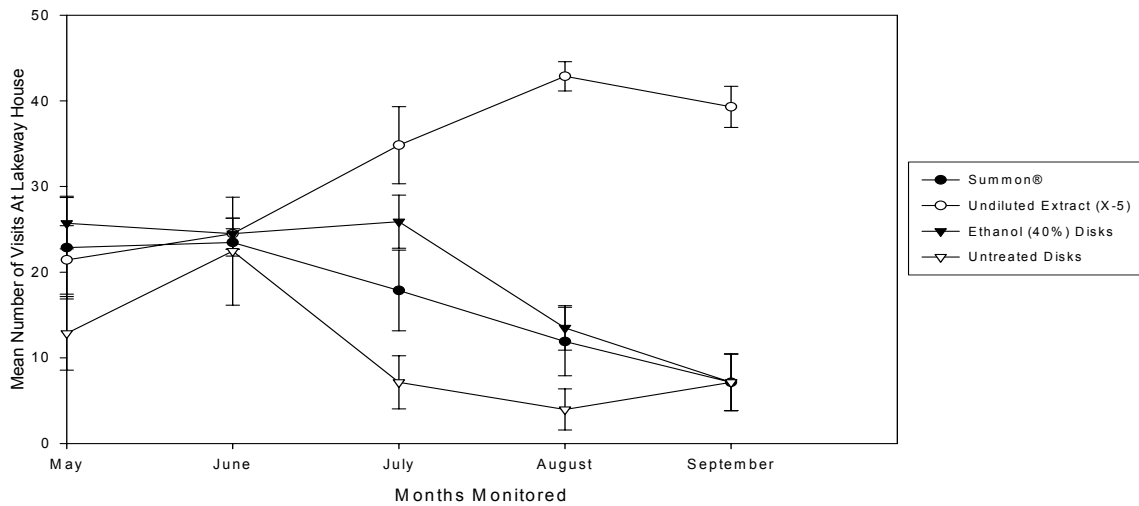
Figure 27. Mean number of visits per month by *R. flavipes* discovered at the various treatments around the Lakeway House at thirty-one different time periods from May 16th through September 18, 2003.

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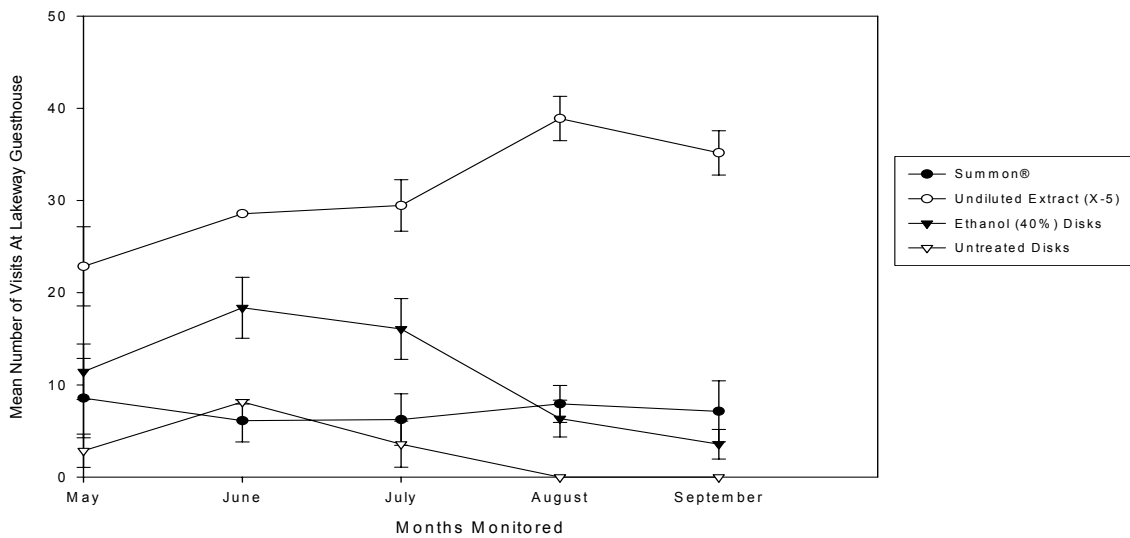
Figure 28. Mean number of visits per month by *R. flavipes* discovered at the various treatments around the Lakeway Guesthouse at thirty-one different time periods from May 16th through September 18, 2003.
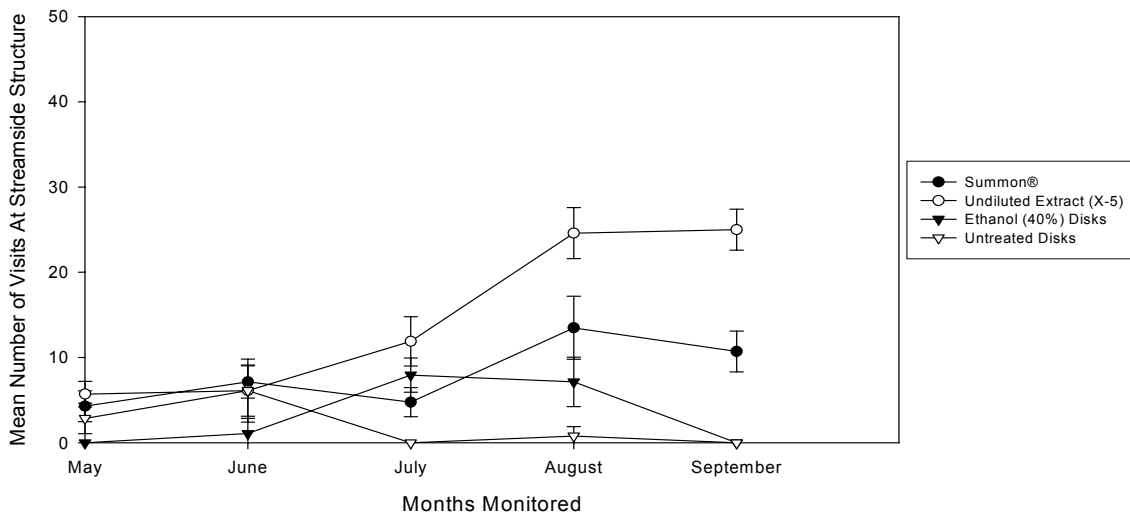
Figure 29. Mean number of visits per month by *R. flavipes* discovered at the various treatments around the Streamside House at thirty-two different time periods from May 16th through September 18, 2003.
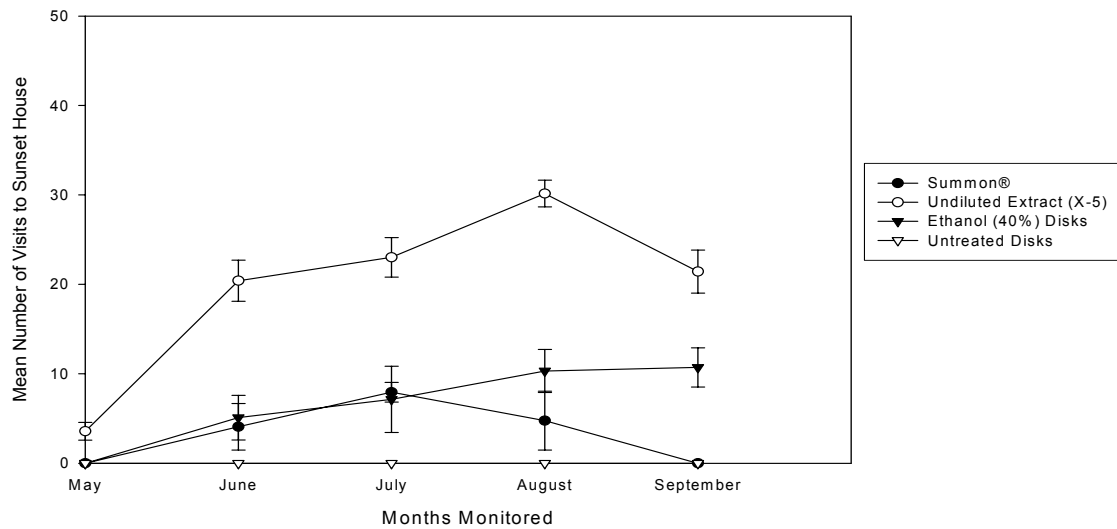
Figure 30. Mean number of visits per month by *R. flavipes* discovered at the various treatments around the Sunset House at thirty-one different time periods from May 21st through September 18, 2003.
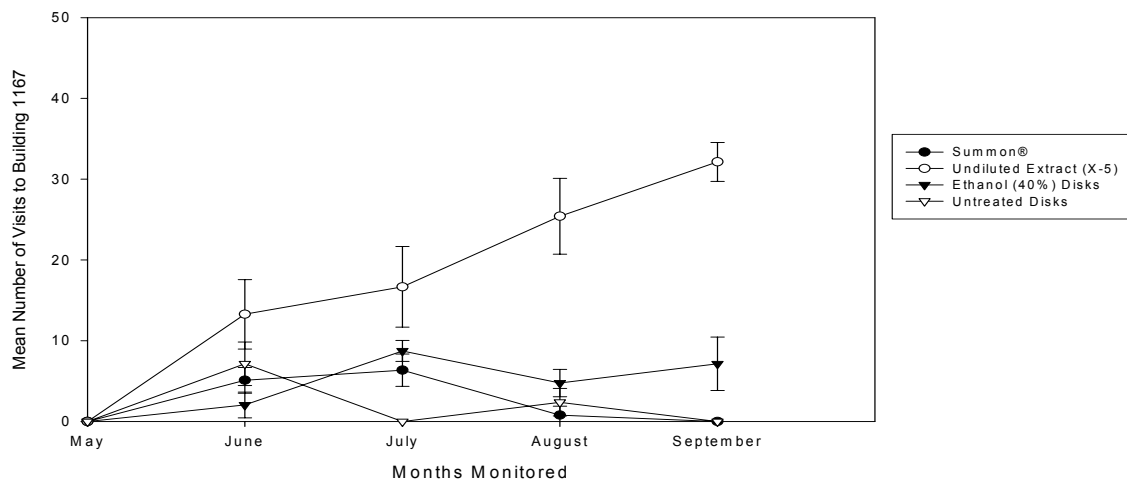
Figure 31. Mean number of visits per month by *R. flavipes* discovered at the various treatments around Building 1167 at twenty-seven different time periods from May 30th through September 18, 2003.

The monitoring stations in the field plot were monitored thirty-one times. In the field

plot, the most termite visits were observed in the *M. anisopliae* ethanol extract (X-5)

treated monitors, which differed significantly different (p≤ 0.05) from other treatments

through time. The Summon® treated monitors were significantly different from

untreated controls, and showed slight recruitment during the summer months, but

declining recruitment in the fall. Both the ethanol treated monitors and the untreated

monitors were the least preferred treatments. The mean number of visits observed in the

field plot for each treatment and for each time period monitored is shown in Figure 32.
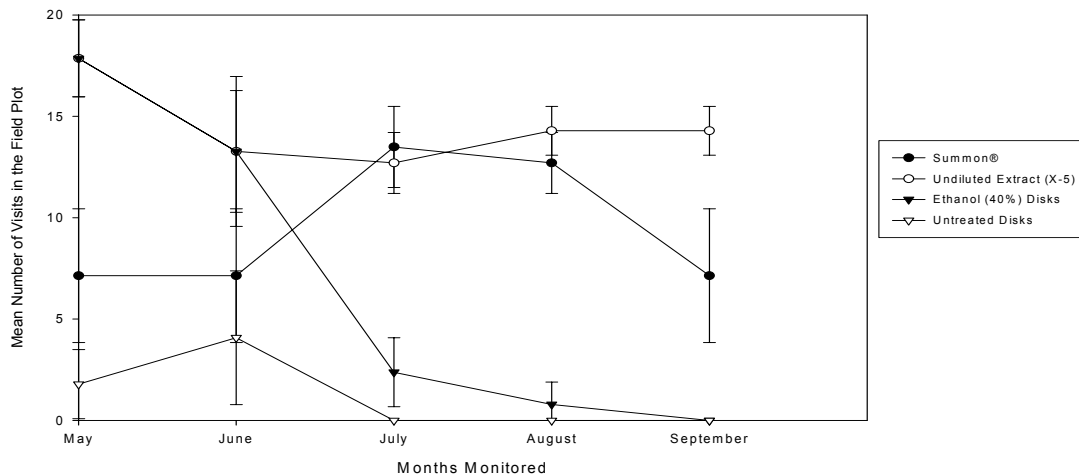
Figure 32. Mean number of visits per month discovered at the various treatments within the Field Plot at thirty-one different time periods from May 21st through September 18, 2003.

DISCUSSION

**Olfactometer:** To test the attractancy of *M. anisopliae*, choices of sterile rice or sterile corn and differing generations of *M. anisopliae* mycelia were given to *C. formosanus* and *R. flavipes*. There were no published studies testing the attractancy of mycelium or mycelial extract of *M. anisopliae* in an olfactometer, prior to this research.

Fungal matrix or sterile rice or sterile corn were presented to groups of three hundred termites, *C. formosanus* was more mobile than *R. flavipes*, in addition to having a larger colony size and being more aggressive (Cornelius and Osbrink, 2001). According to Zimmerman (1993), *M. anisopliae* attracts termites, and we were able to demonstrate this when testing mycelia. Both termite species showed a preference for fungal mycelia or ethanol extracts as compared to sterile rice or sterile corn. Significantly more *R. flavipes* were attracted to the second (C16) generation of *M. anisopliae* cultured on corn than the first generations of *M. anisopliae* cultured on corn (C15) or rice (FF1000). For *C. formosanus*, there were equal numbers of termites that preferred the first generation of *M. anisopliae* cultured on rice (FF1000) and the second generation of *M. anisopliae* cultured on corn (C16).

The relative attractancy of *M. anisopliae* extract and Summon®, which is marketed by FMC as a preferred food source for termites, was also tested in the olfactometer. Currently, Summon® is being used in monitoring systems as a preferred food source to enhance feeding by termites in commercial, termite baiting systems. A choice was given to *R. flavipes* of *M. anisopliae* extract treated fiber pulp disks at four different

concentrations, a Summon® disk, and an untreated fiber pulp disk. When the treated

materials were presented to groups of three hundred termites, the termites showed a

consistent preference of *M. anisopliae* extract treated disks over Summon® and untreated

disks.

Although both termite species were attracted to the fungal treatments, as opposed to

the controls, the testing environment was completely artificial. In this experiment,

termites were in constant light and had to travel against the air current moving through

the plastic tubes of the olfactometer before reaching the fungal treatments. In nature,

termites live underground and are rarely exposed to outside air. Nonetheless, by using

of an olfactometer we could measure and record the response of the termites to

treatments.

Glass Tube Bioassays: Tunneling distances in bioassay tubes were measured to

evaluate the attractancy of the fungal matrix to both *R. flavipes* and *C. formosanus.*.

Both *R. flavipes* and *C. formosanus* showed a maximum amount of tunneling within

control tubes.

The majority of tunneling occurred within the first 24 hours, but testing continued for

6 days. The experimental design (Su *et al.*, 1993; Gold *et al.*, 1994; Gold *et al.*, 1996;

and Waite *et al.*, *In Press*) was effective at measuring tunneling distances and responses

of each termite species to different substrates on which the fungal mycelia were cultured.

Overall, *C. formosanus* tunneled farther than did *R. flavipes*. When termites were placed

on top of the bioassay tubes and allowed to tunnel, there was a greater amount of

tunneling by both species into the first (C15) and second (C16) generation of *M.*

anisopliae cultured on corn as compared to the first generation of *M. anisopliae* cultured on rice (FF1000). The termites tunneled to the inoculated rice and remained in the treatment areas. The *M. anisopliae* grown on the rice substrate was acceptable to the foraging termites, since they remained in the mycelium and never tunneled the remaining distance to the end of the tube.

The goal of any termite management program is to reduce colony size to a level that is not significantly damaging to a structure. The mortality of *R. flavipes* and *C. formosanus* six days after being exposed to three different generations of fungi aged for 12 weeks was evaluated. In a recent report by Susan Dean (Annual Research Progress Report to Ideasphere, Inc. on 4/10/03), is consistent with the mortality we found in this study. The results indicated that the mortality caused by fungi was not dependent on the substrate on which the fungal strains were cultured. For *R. flavipes,* the lowest amount of mortality was observed in the first generation of *M. anisopliae* cultured on rice (FF1000), and the highest mortality was observed in the second (C16) and first (C15) generations of *M. anisopliae* cultured on corn, respectively. For *C. formosanus*, the lowest mortality was observed in the second generation of *M. anisopliae* cultured on corn (C16) and the highest mortality was observed in the first generation of *M. anisopliae* cultured on rice (FF1000) and the first generation of *M. anisopliae* cultured on corn (C15), respectively. Although termite mortality of the termites was relatively low (36%), it resulted for up to 12 weeks without refrigeration. In terms of commercialization, this would correspond with current methods of servicing pest control accounts on a quarterly basis.

Glass Plate Bioassay Study: Similar thin layer approaches of Su and Puche (2003) were used to test the attractancy of different dilutions of *M. anisopliae* extract to *R. flavipes,* compared to Summon® marketed by FMC as a preferred food source for termites. The number of termites observed daily around each treatment disk was an indication of acceptability to the termites. We found that this experimental technique allowed termites to be monitored, and we were able to record their daily movements. The results indicate that *R. flavipes* was more attracted to various dilutions (1:0, 1:10, 1:100, 1:1000) of *M. anisopliae* extracts on disks than to Summon®, ethanol treated disks, and untreated disks. There was an inverse relationship between the concentration of ethanol extract dilutions of *M. anisopliae* (X-5) and the number of termites observed at each treatment. The undiluted extract was apparently too concentrated and may have acted as a deterrent to attractancy as compared to dilutions of the extract. All of the dilutions became more appealing to the termites over time, perhaps due to a dilution effect from the addition of water every 48 hours. Also, there was evidence that the X-5 extract of *M. anisopliae* recruited other termites to the treated disks. Summon® was highly acceptable to foraging termites, but there was no evidence of recruitment in these tests.

The amount of consumption of cellulose matrix was measured for each treatment in the glass plate bioassays and served to indicate how acceptable the treatments were to termite test populations. The results indicated that *R. flavipes* fed on *M. anisopliae* extract treated disks (X-5), but feeding was concentration dependent. After 12 days of exposure within the glass plates, the interaction of the dilution and treatment was

significantly correlated to the final weight of the test disks. The greatest consumption by termites was found in the Summon®, and the least amount in the 1:1000 dilution of *M. anisopliae* extract treated disks. These results indicated that extracts of *M. anisopliae* (X-5) were attractive, but were not phagostimulants. Summon®, on the other hand, was neutral in attractiveness but promoted feeding once the disks were located by foraging termites.

Field Evaluation: The attractancy of the undiluted *M. anisopliae* extract was compared to Summon® in field plots and around termite infested urban structures. Attractancy was measured by the presence or absence of termites in commercial termite monitors. At the beginning of the study, termites showed no preference to treatments in either the field plot or around the urban structures. It was difficult to correlate the results of the field tests with those conducted in the laboratory, since we were unable to control the environmental conditions of temperature, rainfall, and seasonality of termite foraging in the field (Houseman *et al*., 2001). However, the general activity observed within the bait matrix increased between the last week in June and the first week in July. This increase may have been caused by rain showers that fell on June 30th and July 3rd, 6th and 7th. These showers allowed the termites to tunnel faster and with more ease through the soil. This correlates to the seasonal patterns of foraging termites that Houseman *et al*. (2001) observed. After eight weeks, however, the termites were more attracted to the *M. anisopliae* extract treated monitors found around the urban structures and in the field plot. Overall, there was a significant difference between the extract treated monitoring stations and other treatments, with evidence of recruitment through time.

In the field testing, *R. flavipes* were presented with a choice of feeding on treated or nontreated termite monitors. The least attractive treatment observed in both the field plot and the structures were the untreated disks, which resulted in the lowest number of termite visits. However, when the undiluted *M. anisopliae* ethanol extract (X-5) was added to the monitoring system, these monitors showed a trend of increasing termite visits as compared to the untreated, ethanol treated, and Summon®. However, when higher concentrations of fungal extracts were apparently repellent to termites, perhaps rendering the monitoring system inefficient. Some lower concentrations of fungal extract were attractive to termite foragers, increasing the likelihood of termites feeding on the treated matrix. Any increase in attractancy or consumption of the matrix would potentially increase the effectiveness of monitoring stations. Even though the use of fungal extracts or Summon® may increase termite feeding at the monitors, it must be remembered that the purpose of termite pest management is to protect the structure not to merely attract termites to termite monitors.

SUMMARY AND CONCLUSIONS

The first objective of this study was to determine if the mycelia mat matrix of *M. anisopliae* was attractive to *R. flavipes* and *C. formosanus* using an olfactometer. This study focused on determining if the fungus emitted volatiles to which other termites would respond. *M. anisopliae* was attractive to both *R. flavipes* and *C. formosanus* when compared to untreated matrix controls. Both species of termites showed different levels of attractiveness depending on the generation and age of *M. anisopliae,* which they were exposed in the olfactometer. Overall, *C. formosanus* showed more movement towards the treatments in the olfactometer than did *R. flavipes*. This was apparently due to the termites moving from the center chamber and traveling to the radiating dishes, but failing to leave the radiating dishes after their arrival.

 The effect of an ethanol extract of *M. anisopliae* (X-5) was also observed in the olfactometer to test preference by *R. flavipes* for different dilutions of the fungal extract on treated disks, compared to an ethanol (40%) treated disk and Summon®. The 1:1000 dilution of *M. anisopliae* extract and Summon ® was strongly preferred over the other treatments, and the 1:10 dilution was the least attractive treatment.

The second objective was to determine tunneling distances by *R. flavipes* and *C. formosanus,* when exposed to aged strains of *M. anisopliae*. When both species of termites were exposed to different generations of *M. anisopliae* they showed varying degrees of attractancy and mortality. There was variation among the three generations of *M. anisopliae* as well as among different statues and ages of mycelia. When the

tunneling distances of both species of termites were analyzed, greater tunneling was displayed in the first (C15) and second (C16) generations of *M. anisopliae* cultured on corn compared to the first generation of *M. anisopliae* cultured on rice (FF1000). Neither species was inhibited by the presence of the first and second generations of *M. anisopliae* cultured on corn, permitting them to tunnel all the way through the bioassay tubes. However, the first generation of *M. anisopliae* cultured on rice caused both species of termites to stop tunneling and live within the fungal matrix, rather than tunnel farther in the bioassay tubes.

The third objective was to determine if the fungal mycelium caused termite mortality in the bioassay tubes. The goal of any termite treatment is to reduce the colony to a point that will not cause serious damage to a structure or eliminate the colony completely. Observing the mortality of *R. flavipes* and *C. formosanus* after being exposed to the fungal matrix in the bioassay tubes for six days permitted us to conclude that some strains of the fungi adversely affected termite survival. The lowest mortality was observed in the soil, sterile rice and sterile corn controls for both species. There was a mortality effect on the termites when the fungi were aged. As the three strains of *M. anisopliae* were aged for 12 weeks, *R. flavipes* the lowest amount of mortality was observed in the first generation of *M. anisopliae* cultured on rice (FF1000), and the highest mortality was observed in the second (C16) and first (C15) generations of *M. anisopliae* cultured on corn, respectively. For *C. formosanus*, the lowest mortality was observed in the second generation of *M. anisopliae* cultured on corn (C16) and the

highest mortality was observed in the first generation of *M. anisopliae* cultured on rice (FF1000) and the first generation of *M. anisopliae* cultured on corn (C15), respectively.

The fourth objective was to determine if *R. flavipes* was attracted to the ethanol extract of *M. anisopliae* (X-5) and a commercial preferred food source (Summon®), and to estimate the percent consumption of the cellulose matrix by the termites. The ethanol extract of *M. anisopliae* (X-5) was tested using glass plates bioassays to determine preference and amount of consumption by *R. flavipes*, when given a choice of untreated fiber kraft pulp disks, an ethanol (40%) treated disks, Summon®, and different dilutions (1:0, 1:10, 1:100, 1:1000) of *M. anisopliae* ethanol extract (X-5) treated disks. The termites showed a strong preference to the 1:1000 dilution of the fungal extract. Summon® was the least preferred treatment and did not elicit recruitment. There was a trend for increased recruitment to all ethanol extract (X-5) treatments. One possibility for this observation was the dilution of the extract became more acceptable to the termites, as degradation of the chemical compounds occurred over time. The lower concentrations were most attractive, and there was an inverse relationship based on concentration through time.

An analysis of the consumption of test cellulose matrix showed that while Summon® was not attractive, it was very palatable. The ethanol extract (X-5) of *M. anisopliae* was attractive, but was not a phagostimulant. Summon® is composed of a matrix of ground cellulose materials that is formed into a cookie. There are no adhesive products holding the cookie together, so it is possible that the cookie could have disintegrated when 4 ml of water was added every two days. Also, Summon® appeared to be readily consumed

by the termites as compared to fiber kraft pulp treatments. Another possibility in regards to a greater number of termites found around the extract treatments, but not consuming large amounts of these treatments, is that extract treatments elicit visitation but not consumption. It was apparent that attractancy and consumption are not necessarily related to each other.

The undiluted ethanol extract of *M. anisopliae* (X-5) was placed in termite monitors around existing urban structures and in a field plot. There appeared to be recruitment by other termites to the extract treated monitors through time. Overall, the most termite visits occurred in the ethanol extract of *M. anisopliae* (X-5) treated monitors, and the fewest in monitors containing untreated fiber kraft pulp disks. The most termite visits found for the undiluted extract were around Lakeway Manor House and Guest House. This could be due to a frequent watering routine that would dilute the extract and make it more attractive to *R. flavipes*.

Summon® were also placed in bait stations to determine the effect of this commercial product in natural urban environments. FMC claims that subterranean termites preferred Summon® when they forage normally, but are not attracted to the product from far distances. This correlates with the findings in this study, since Summon® did not recruit other termites but was a palatable food source to the termites.

Since the undiluted extract emits volatiles in the soil, it can be viewed as a possible termite attractant. The most effective and time efficient treatment for termite control would be to use Summon® in conjunction with the ethanol extract of *M. anisopliae* (X-5). Since the extract is an attractant and the Summon® is a preferred food source, the

termites would be attracted to the termite monitor and continue to feed within the monitor. This would allow the termites to feed on the active ingredient and distribute it to the rest of the population. This research has yet to be done.

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APPENDIX A

MEAN INITIAL AND FINAL WEIGHTS AND AVERAGE CONSUMPTION

Appendix Table 1. Mean initial and final weights and average consumption for glass plate bioassays containing undiluted ethanol extract *M. anisopliae*.

Treatments	Weight (mg) At Day 0 (Mean ± Std. error)	Sample Size (n)	Weight (mg) At Day 12 (Mean ± Std. error)	Sample Size (n)	Difference In Weight (Mean ± Std. error)	Sample Size (n)	Percentage Consumed of The Treatment
Undiluted Extract	1750 ± 623	20	1256 ± 154	5	221±129	5	17.4%
Summon Disks	1590 ± 620	20	1288 ± 73	5	302 ± 73	5	19.1%
Ethanol Treated Disks	1750 ± 623	20	1620 ± 37	5	116 ± 28	5	6.6%
Untreated Disks	1750 ± 623	20	1524 ± 134	5	221 ± 62	5	12.6%

Appendix Table 2. Mean initial and final weights and average consumption for glassplate bioassays containing 1:10 dilution of ethanol extract *M. anisopliae*.

Treatments	Weight (mg) At Day 0 (Mean ± Std. error)	Sample Size (n)	Weight (mg) At Day 12 (Mean ± Std.error)	Sample Size (n)	Difference In Weight (Mean ± Std. error)	Sample Size (n)	Percentage Consumed of The Treatment
1:10 Dilution of Extract	1750 ± 623	20	1466 ± 51	5	284 ± 51	5	17.4%
Summon Disks	1590 ± 620	20	1180 ± 32	5	410 ± 53	5	25.8%
Ethanol Treated Disks	1750 ± 623	20	1558 ± 50	5	192 ± 50	5	10.9%
Untreated Disks	1750 ± 623	20	1660 ± 24	5	106 ± 24	5	6.1%

Appendix Table 3. Mean initial and final weights and average consumption for glassplate bioassays containing 1:100 dilution of ethanol extract *M. anisopliae.*

Treatments	Weight (mg) At Day 0 (Mean ± Std. error)	Sample Size (n)	Weight (mg) At Day 12 (Mean ± Std. error)	Sample Size (n)	Difference In Weight (Mean ± Std. error)	Sample Size (n)	Percentage Consumed
1:100 Dilution of Extract	1750 ± 623	20	1484 ± 51	5	266 ± 78	5	15.2%
Summon Disks	1590 ± 620	20	1110 ± 53	5	480 ± 53	5	30.2%
Ethanol Treated Disks	1750 ± 623	20	1584 ± 30	5	166 ± 29	5	9.5%
Untreated Disks	1750 ± 623	20	1662 ± 29	5	88 ± 29	5	5.1%

Appendix Table 4. Mean initial and final weights and average consumption for glassplate bioassays containing 1:1000 dilution of ethanol extract *M. anisopliae.*

Treatments	Weight (mg) At Day 0 (Mean ± Std. error)	Sample Size (n)	Weight (mg) At Day 12 (Mean ± Std. error)	Sample Size (n)	Difference In Weight (Mean ± Std. error)	Sample Size (n)	Percentage Consumed
1:1000 Dilution of Extract	1750 ± 623	20	1612 ± 15	5	138 ± 15	5	7.9%
Summon Disks	1590 ± 620	20	962 ± 89	5	628 ± 89	5	39.5%
Ethanol Treated Disks	1750 ± 623	20	1608 ± 29	5	142 ± 29	5	8.1%
Untreated Disks	1750 ± 623	20	1598 ± 28	5	152 ± 28	5	8.7%

APPENDIX B

MEAN NUMBER OF VISITS

Appendix Table 5. Mean percentage of visits at each bait treatment for each month from May-September around all 5 structures.

Location	Treatments	May	June	July	August	September
Streamside	Summon	4.3%	7.1%	4.8%	13.5%	10.7%
	Extract	5.7%	6.4%	11.9%	24.6%	25.0%
	Ethanol	0.0%	4.3%	7.9%	7.1%	0.0%
	Untreated	2.9%	6.4%	0.0%	0.8%	0.0%
Lakeway House	Summon	22.9%	23.5%	17.9%	11.9%	7.1%
	Extract	21.4%	24.5%	34.8%	42.9%	39.3%
	Ethanol	25.7%	24.5%	25.7%	13.5%	7.1%
	Untreated	12.9%	22.5%	7.1%	4.0%	7.1%
Lakeway Guesthouse	Summon	8.6%	6.1%	6.3%	7.9%	7.1%
	Extract	22.9%	28.6%	29.5%	38.9%	35.7%
	Ethanol	11.4%	18.4%	16.1%	6.4%	3.6%
	Untreated	2.9%	8.2%	3.6%	0.0%	0.0%
Sunset	Summon	0.0%	4.1%	7.9%	4.8%	0.0%
	Extract	3.6%	20.4%	23.0%	30.2%	21.4%
	Ethanol	0.0%	5.1%	7.1%	10.32%	10.7%
	Untreated	0.0%	0.0%	0.0%	0.0%	0.0%
Building 1167	Summon	0.0%	5.1%	6.4%	0.79%	0.0%
	Extract	0.0%	13.3%	16.7%	25.4%	32.1%
	Ethanol	0.0%	2.0%	8.7%	4.8%	7.1%
	Untreated	0.0%	7.1%	0.0%	2.4%	0.0%

Appendix Table 6. Mean percentage of visits at each bait treatment for each month from May-September found in the field plot.

Location	Treatment	May	June	July	August	September
Field Plot	**Summon**	7.1%	7.1%	13.5%	12.7%	7.1%
	Extract	17.9%	13.3%	12.7%	14.3%	14.3%
	Ethanol	17.9%	13.3%	2.4%	0.79%	0.0%
	Untreated	1.8%	4.1%	0.0%	0.0%	0.0%

VITA

Kimberly M. Engler was born on May 5, 1978 in New Braunfels, Texas to Bonnie and Kyle Engler, both of New Braunfels, Texas. She lived in New Braunfels her whole life, before she started college. She has had the opportunity to visit Italy, Mexico, and the Bahamas. She received her B.A. in biology from Texas A&M University in December of 2000. In May 2004, she received her Master of Science degree in entomology from Texas A&M University. During her master's program, she has gained interest in different aspects of entomology including urban pest management, medical entomology, and toxicology.

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