LABORATORY AND FIELD EVALUATION OF IMIDACLOPRID AGAINST *RETICULITERMES FLAVIPES* (KOLLAR) AND *COPTOTERMES FORMOSANUS* SHIRAKI (ISOPTERA: RHINOTERMITIDAE) SUBTERRANEAN TERMITES IN TEXAS

A Thesis

by

TONY CHRISTOPHER KEEFER

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 2010

Major Subject: Entomology

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Approved by:

Chair of Committee,	Roger Gold
Committee Members,	Jimmy Olson
	Gary Briers
Head of Department,	Kevin Heinz

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ABSTRACT

Laboratory and Field Evaluation of Imidacloprid against *Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) Subterranean Termites in Texas. (May 2010)

Tony Christopher Keefer, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Roger Gold

In one study described herein, 20 privately owned structures were treated with a 0.05% application of imidacloprid (Premise® 75 WSP) in order to control infestations of subterranean termites. All applications were made at 15 L per 3.05 linear m per 0.30 m of depth. Ten structures were infested with *Reticulitermes flavipes* (Kollar) and ten structures were infested with *Coptotermes formosanus* Shiraki. All structures were inspected through 42 months post-treatment. Only one structure infested with *R. flavipes* required post-treatment action. Six structures infested with *C. formosanus* required post-treatment action.

In another study, efficacy data were gathered on Premise® Granules when broadcast over an open field and when utilized as a "spot treatment" for control of subterranean termites infesting structures. Open field grids with active *R. flavipes* were utilized in this study. Grids measuring 8.53 m x 7.32 m were marked off, in-ground commercial termite monitors were installed, and grids were treated with Premise® Granules. Untreated southern yellow pine surface boards were then placed in grids to

determine if Premise® Granules would suppress foraging and feeding on surface boards. Premise® Granules did suppress surface feeding of *R. flavipes* for 9 months post-treatment, although termites were active throughout the study in in-ground commercial termite monitors within treated grids.

In a third study, 10 structures built on monolithic slabs, five received a “spot treatment” with Premise® Granules at points of subterranean termite infestation 0.61 m either side of active exterior subterranean termite mud tubes. Structures were inspected through 12 mo post-treatment. Suppression of *R. flavipes* was sustained for 8 wks in all treatment replications following application of granules, with failures at 8, 12, and 28 weeks post-treatment.

A laboratory trial was initiated to simulate field treatments with Premise® 75 WP 0.10 % AI imidacloprid for treatments of structures. The focus of this research was to investigate the dissipation and translocation of imidacloprid in urban environments. Treated sandy loam soil was added to 19-L buckets. Four different plant species commonly found in urban environments were planted in buckets. Results in these trials indicate that imidacloprid was soluble and that there is leaching.

DEDICATION

This thesis is dedicated to my daughters Becca and Molly. I love you both with all of my heart and soul.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Roger Gold, and my committee members, Dr. Jimmy Olson, and Dr. Gary Briers, for their guidance and support throughout the course of this research.

Thanks also go to everyone at the Center for Urban and Structural Entomology; the past few years have been a pleasure because of you: Dr. Robert Puckett and Dr. James Austin for your advice, Laura Nelson for your countless hours of scheduling my trips and then re-scheduling my trips, Bill Summerlin for your smiling face. I want to say a special thanks to Dr. Jason Meyers, Dr. James Austin and Dr. Robert Puckett for always providing a constant source of humor, no matter what the situation. I would not have been able to get through this without you.

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1. INTRODUCTION

Termites belong to the order Isoptera (Haverty 1976). Termites are wood-destroying insects that can cause serious damage to man-made structures, live trees, and selected crops. They have been described in every state in the United States except Alaska (Su et al. 2001, Austin et al. 2005). Of the over 2300 termite species in the world, 183 are known to damage structures; and 83 species cause other significant damage (Edwards and Mills 1986, Su and Scheffrahn 1998). In recent years, urban sprawl has contributed greatly to the economic impact of termites in the United States (Su and Scheffrahn 1990, 1998).

Termites are eusocial insects and live in colonies made up of a caste system. Eusocial is characterized by two generations of conspecific adults living together (a colony) and cooperating with each other to the extent that a strong reproductive skew is observed (Wilson 1971, Higashi et al. 2000). The caste is made up of the following; workers, soldiers, immature, and functional reproductives (Krishna and Weesner 1969, Thorne 1996). The worker caste, also known as pseudergates, is usually the most abundant within the colony (Grasse´ and Noirot 1947, Wilson 1971). The duties of workers include foraging, nest excavation, and feeding of soldiers, larvae, and reproductives. Soldiers make up a small portion of the colony and their only duty is colony defense. They possess large, sclerotized head capsules with mandibles used to defend the colony against invaders (Krishna 1969). In *Reticulitermes* there is no true worker caste. Apterous nymphs also called pseudergates, perform colony maintenance.

This thesis follows the style of Journal of Economic Entomology.

Immature individuals can develop into any of the three castes. Their developmental fate is controlled by chemical cues from the colony and the environment (Krishna and Weesner 1969).

There are seven common genera of subterranean termites found in North America including: *Amitermes, Anoplotermes, Coptotermes, Gnathamitermes, Heterotermes, Reticulitermes* and *Tenuirostritermes. Reticulitermes* is the most widespread with species found throughout North America. The genus *Reticulitermes* includes *R. flavipes* (Kollar), *R. tibialis* Banks, *R. virginicus* (Banks), *R. hageni* Banks, *R. hesperus* Banks, *R. okanagenensis, R. malletei,* and *R. arenicola* Goellner. *Reticulitermes flavipes* is the dominant subterranean termite species found throughout the United States and is responsible for most damage to structures done by this genus of subterranean termites. *Reticulitermes flavipes* is known as the Eastern subterranean termite.

Currently there are four species of *Reticulitermes* found in Texas. Documented *Reticulitermes* species found in Texas include *R. flavipes, R. tibialis, R. virginicus,* and *R. hageni* (Howell et al. 1987). *Reticulitermes flavipes* is found throughout the state of Texas and is the dominant species in relation to structural damage. Its peak swarming times in Texas are generally from late February to early April (Furman 2000). The alates or winged swarmers have dark brown to black bodies. Their wings are approximately 10 mm in length and are considered translucent. The soldiers are characterized by a large rectangular-shaped head with large mandibles. These mandibles have no internal teeth and curve inward at the proximal tip (Messenger 2002).

There are at least two species of *Coptotermes* of subterranean importance in North America, both of which are invasive. Like most exotic introduced pest species without natural enemies or indigenous competing factors, these particular pests severely and irrevocably altered landscapes. *Coptotermes formosanus* Shiraki is found throughout the Gulf Coast region from Florida to Texas, and *C. gestroi* is found on peninsular Florida (Scheffrahn and Su 2005). C*optotermes formosanus* is also known as the Formosan subterranean termite. Today, *C. formosanus* is found in 30 counties in Texas, and is likely to continue spreading throughout the state via interstate commerce. In Texas, *C. formosanus* was originally reported from the city of Beaumont in 1962 (W. Clark personal communication 2010). This termite is of great concern because it causes significant damage to structures, a variety of wood products, and a number of trees species (La Fage 1987, Su and Tamashiro 1987). *Coptotermes formosanus* is of great concern due to its large colony sizes, which can number in the millions, and their voracious foraging behavior (Su and Tamashiro 1987, Su and Scheffrahn 1998, Morales-Ramos and Rojas 2001). Their presence in hurricane-prone regions of the United States Gulf Coast is of major concern due to the severe damage they cause to living trees which can then fall, producing damage to property and injury to people during wind events (La Fage 1987, Morales-Ramos and Rojas 2001). The peak swarming time for *C. formosanus* in Texas is generally in May through late June at dusk (Furman 2000). The alates or winged swarmers typically have dark brown heads with body and pronotum being yellowish to dark brown. Their wings are approximately 13 mm in length and are covered with dense hair. The soldiers are characterized by a tear-drop shaped head with

large mandibles. These mandibles have no internal teeth and curve inward at the proximal tip (Messenger 2002).

Many options are currently available to control subterranean termites. The strategy of establishing a complete chemical barrier to protect a structure and the methods for application of such chemical barriers are as pertinent and effective today as they were 50 years ago (Gold et al. 1994,Gold et al. 1996). The application of soil-applied termiticides continues to be the preferred method of control for subterranean termites. Termiticides used in this strategy should be efficacious against all castes to provide an effective barrier (Gatti and Henderson 1996), but subtle differences in susceptibility to termiticides by termites have been detected even within the same species of some genera. Also, significant changes have occurred in what chemicals can be used as barriers against termites and the challenge of controlling these destructive pests remains enormous (Raina et al. 2001). Several new chemical groups have been developed including pyrethroids, phenylpyrazoles, chloronicotinoids and fiproles, to combat termites in recent years.

Experts from the National Pest Management Association estimate the cost to control termites annually in the United States to be $5 billion (NPMA 2005). When the cost of building repair is included, cost estimates can be as high as $11 billion annually in the United States, and as much as $22 billion globally (Su 2002). Today there are many options available to protect structures from subterranean termites.

Termite control measures include, but are not limited to, liquid sub-soil treatments, above and in-ground baiting systems, stainless steel mesh, diatomaceous

earth, insecticide- impregnated polymer barriers, sand, salt, and post-construction applications of chemical made directly to wood (Mampe 1991, Grace and Yamamoto 1993, Robertson and Su 1995). Granular termiticide application to the soil for control of subterranean termites is a relatively new concept that is currently being investigated.

Providing a dependable and effective termite control job is a complex duty. It requires knowledge in many areas including; termite biology, different control tactics available, tools and equipment used, landscape and hydrology surrounding a structure, and building construction (Forschler and Jenkins 2000). In addition, one must be experienced in the identification of termites. This is of major importance because different species of termites may only be susceptible to specific treatments. A termite control expert must also know and be familiar with construction. They must know the basics of general construction as well as the common construction practices within their region. One must also be familiar with common electrical and plumbing practices as they relate to termite entry points. They must also know what tools to use and when and how to operate them to effectively control termites. They must know termite biology and habits of each species of termite in their region that is known to attack structures. Three other important factors to consider when planning a termite treatment are where; food sources are found, suitable moisture levels occur, and which soil types are preferred for termite survival (Suiter et al. 2002). These specific factors are known as conducive conditions.

A current list of registered active ingredients used in termiticides for soil treatments currently regulated by the United States Environmental Protection Agency

(US EPA 2008) include: bifenthrin, cypermethrin, permethrin, chlorfenapyr, acetamiprid, imidacloprid, and fipronil. Bifenthrin, cypermethrin, and permethrin all belong in the family of chemicals known as pyrethroids. Fipronil is the lone member of the fiproles, a relatively "new" class introduced in 1990 (Ware and Whitacre 2004). Chlorfenapyr is a member of the phenylpyrazole family of chemicals (Valles and Koehler 1997). Acetamiprid and imidacloprid are found in the chemical family known as the chloronicotinyls (Abbink 1991, Gahlhoff and Koehler 2001). In cases where a liquid chemical barrier cannot be established, termite baits may be employed for control. Most of the termite baits available for use today are found in the benzoylphenyl urea chemical group and are slow acting insect growth regulators which inhibit metamorphosis, specifically molting.

The newer generations of chemicals have been developed since the demise of the chlorinated hydrocarbon insecticides such as chlordane and lindane are inferior in terms of persistence in the soil, as compared to the old chlorinated hydrocarbon of the past. Chlorinated hydrocarbon compunds such as chlordane were very persistent in the soil lasting up to 50 years. The questions of safety to all non-target animals including humans caused a revolution in the chemistries used in termite management. The use of chlorinated hydrocarbons as pesticides was phased out completely by the Environmental Protection Agency (EPA) in 1988 and the use of new chemical classes as termiticides began. The new classes of chemicals which are not as persistent as the chlorinated hydrocarbons in the environment need to be explored more intensely.

One such chemical is imidacloprid 1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine (Fig 1.), a systemic chloronicotinyl insecticide with a novel mode of action, that acts as an agonist of the nicotinyl receptor (Bai et al. 1991, Mullins 1993). Imidacloprid ($C_9H_{10}ClN_5O_2$) is sold under the trade name Premise® by Bayer Environmental Science (Research Triangle Park, NC).

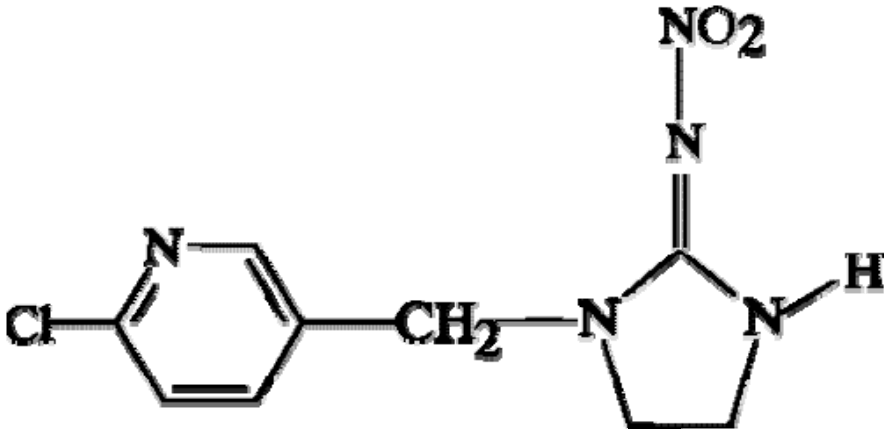

Fig. 1. The chemical structure of imidacloprid (Adapted from Fernandez-Perez et al. 1998).

Imidacloprid is commonly used to control subterranean termites and is available in several formulations including: liquid (Premise® 0.5 SC, Premise® 2, Premise® Pro); wettable powder (Premise® 75WP); gel (Premise® Gel); foam (Premise® Foam) and most recently, as a granule (Premise® Granules). All of these formulations are regulated as termiticides for the control of subterranean termites. Premise® is reportedly a non-repellent termiticide and the liquid; and wettable powder formulations are applied as a soil barrier to foraging subterranean termites, which allows the termites to unknowingly

come in contact with the product, and then transfer the active ingredient back to nestmates via trophallaxis, moving contaminated soil, or by simple grooming.

Imidacloprid was first synthesized in 1985 and was registered in France as an agricultural pesticide to be used on sucking insects attacking sugar beets (Sur and Stork 2003). Imidacloprid is a systemic neonicotinoid insecticide that has been commonly used in agricultural settings since registration. Imidacloprid is a systemic insecticide that is translocated through plant vascular tissues after application and can be carried in the tissues of the plants and thus make the plant toxic to insects (Jeppson 1953 Carretero et al. 2003). Imidacloprid's benefits have recently been documented, and it now has been introduced and is being applied in the urban sector of pest management. One of the benefits of this pesticide is that it may decrease the amount of chemical applied, which could lower exposure and cost in populated urban environments (Jeppson 1953). The use of imidacloprid as an urban pesticide was not realized until 2000. This chemical acts as both a contact and a stomach poison which attacks the insect nervous system by attaching to acetylcholine binding sites, called nicotergenic receptors on the receiving nerve cells (Abbink 1991, Ramakrishnan et al. 2000). Once attachment occurs, and the ligand-gated Na^+ cation channel is opened, and the neuron continually fires with the result being death of the insect (Schroeder and Flattum 1984).

The movement of termiticides like imidacloprid and the bio-availability of the chemical in soil have been of concern for several reasons. If the termiticide is not bound to the soil and is leaching, this could cause potential problems in ground water contamination. Also, movement of a termiticide through the soil results in the product

movement off target, and makes it available to non-target organisms and conversely, less available to the “target” species. Further, if the product is degrading, dissipating, or moving through the soil at high rates, this may deem the product ineffective to control subterranean termites for long periods of time. Movement by imidacloprid through the soil was deemed most worthy of further investigation.

All of the questions pertaining to imidacloprid’s movement in the soil can be explored byusing high-performance liquid chromatography (HPLC) likeas done during this research project. HPLC uses a liquid mobile phase (solvents) to transport and separate the components of a chemical mixture. A typical HPLC system is made up of the following units: a solvent cabinet, vacuum degasser, pumping system, autosampler, and a detector. Solvents (mobile phase) are used in HPLC to move the sample through the system. Common solvents used in HPLC include acetonitrile, methanol, hexane and HPLC-grade water. Solvents are stored in reservoirs in volumes that allow the instrument to operate for 8 h or more depending on volumes required for analysis. A stainless steel or fritted glass filter removes particulate matter from the mobile phases so damage or contamination of the pumping system and column does not occur (Poole and Poole 1997). The pumping system of the HPLC unit is used to provide accurate compositions, flows, and control of the pressure necessary to force the mobile phase through the column. Most HPLC systems are equipped with a vacuum degassing unit to remove dissolved gasses from the mobile phase.

In using an HPLC system, the autosampler and injector delivers the sample to the inlet of the column without interrupting the flow of the mobile phase. The column or

stationary phase is where the separation of the sample occurs (Agilent Technologies 2006). The column is tightly packed with silica and a polymer in the form of small particles. Separation occurs when the sample mixture is differentially distributed between the mobile and stationary phases within the column (Poole and Poole 1997). After separation, the sample flows to the detector, where each component is detected and measured. The detector consists of a series of photosensitive diodes placed side by side. The diode array monitors light that passes through the detector. After passing through the detector, the light is dispersed by a quartz prism onto the surface of the diode array. Each diode will receive the light of a slightly different wavelength. When a given substance is eluted through the detector, it can be compared with standards for identification (Agilent Technologies 2006). The components are measured two-dimensionally as a chromatogram. A chromatogram is a recording on paper that delineates which components of the sample were adsorbed by the detector in the process of chromatography. From the chromatogram one can read the retention time and concentration of the components in the sample. The units of concentration are expressed in milli-absorbance unit (mAU). A typical HPLC instrument with all of its components is depicted in Figure 2.

Fig. 2. HPLC with all components: solvent cabinet, vacuum degasser, quaternary pump, autosampler, column compartment, and diode array detector (DAD).

In light of the need for more in depth research on imidacloprid termiticide as a control option for subterranean termites in Texas, the study described herin were performed. The primary goals of this research were to: (1) determine the effectiveness of a flowable formulation of imidacloprid (Premise ® WSP 75 0.05% AI) for control of *R. flavipes* and *C. formosanus* in infested structures in the field;(2) determine the effectiveness of a granular formulation of imidacloprid (Premise® Granules 0.50 % AI) for control of *R. flavipes* in structures and in open field settings; and (3) determine mobility and dissipation of imidacloprid (Premise® 75WP 0.05 % AI) in soils held in laboratory conditions.

The null hypotheses for this research were as follows: (1) there are no significant mortality factors caused by Premise® 75 WSP 0.05% AI between *R. flavipes* and *C. formosanus* subterranean termites in field treatments; (2) there are no significant mortality factors caused by Premise® Granular formulation at 0.5% AI on *R. flavipes* subterranean termites as compared to untreated controls; and (3) there are no significant differences between imidacloprid (Premise® 75 WP 0.05% AI) and untreated controls based on watering (leaching), and degradation due to biotic activity and moisture in the soil.

2. MATERIALS AND METHODS

2.1 Perimeter Treatment Study of Premise® 75 WSP

For purposes of this study, 10 structures infested with Eastern subterranean termites, *Reticulitermes flavipes* and 10 structures infested with Formosan subterranean termites, *Coptotermes formosanus* were selected. Soldier caste termites were collected from all 20 structures and identified with termite identification keys (Scheffrahn and Hope 1996). The structures all had monolithic slab foundations, and had not been treated for subterranean termites during the prior 12 months, as verified through an interview with property structure owner. A diagram of each structure was completed to include all known points of subterranean termite infestation, and all known plumbing and utility penetrations through the slab. Active termite mud tubes were documented from each structure relative to a permanent benchmark such as the corner of the foundation. Each infested structure had a minimum of one active mud tube leading from the soil into the structure. The mud tubes used were located on either an external or internal surface of each structure, and its location had to be such that it was accessible for inspection during repeated visits to the structure. Representative termite specimens were collected and stored in 100% ethanol from all 20 sites as voucher specimens. If termites were eliminated after initial treatment, but then re-appeared at a later date, termites were again collected and stored in 100% ethanol, and were stored as voucher specimens. Each infested structure was inspected at 1, 2, 3, 6, 9, 12, 18, 24, 30, 36 and 42 months post-treatment.

Under supervision of staff from the Center of Urban and Structural Entomology at Texas A&M University, all infested structures were treated by a licensed pest control company with the appropriate dilution (0.05% AI) of Premise® 75 WSP. At each of the structures, one half of the desired volume of water was first added to the pest control operator's tank and then the appropriate amount of Premise® 75 WSP was introduced into the tank, and the remaining volume of water was added to ensure thorough mixing of the solution. The following parameters were used for treatment of all the structures as necessary:

1. An application of a full-volume treatment of Premise® 75 WSP (15 L per 3.05 linear m per 0.30 m of depth) at 0.05% AI around the outside perimeter of the foundation wall by trenching, or by trenching and rodding to a depth of no more than 0.61 m to depth of foundations;
2. A sub-slab injection of Premise® 75 WSP at 0.05% AI extending a minimum of 0.61 to 0.91 m on either side of known infested sites at expansion joints or cracks in slabs. This treatment was performed by down drilling through the slab and making a full-volume application (15 L per 3.05 linear m per 0.30 m of depth). All patios and sidewalks adjacent to structures were down drilled on 30.48 cm centers;
3. A sub-slab injection of Premise® 75 WSP at 0.05% AI was made at or near utility penetrations with known infestations. This treatment was made by drilling through the slab and making application at a rate of 3.77 L of solution per 0.30 m^2; and

4. Premise® 75 WSP at 0.05% AI was applied at a rate of 3.77 L of finished solution per 0.30 m^2 in the exposed soil in bath traps.

Areas with any persistent or reocurring termite activity were re-treated with Premise® 75 WSP at 0.05% AI using the same type of application techniques as were described in the original treatments.

One of the 20 subterranean termite infested structures was treated by a Certified Applicator licensed in the termite category by the Structural Pest Control Service of Texas from ABC Pest and Lawn of College Station, TX . This structure was located in Bryan, TX and was infested with *R. flavipes*. Nine structures that were infested with *R. flavipes*, and one structure infested with *C. formosanus* were treated by a Certified Applicator from Budget Pest Control of Pearland, TX. Nine structures that were infested with *C. formosanus* were treated by a Certified Applicator from Albin Exterminating, Rockport, TX. A flat-blade pick and 10 cm (4 in) shovel were used to dig trenches at all structures. A 50 gallon Continental Belton fiberglass tank (Belton, TX) having an air gap for back flow prevention, and equipped with a constant jet agitation and a HyproD-30 diaphragm pump (Italy) was used for all applicable applications. A JD-9 gun was utilized to deliver termiticides when applicable. When sub-slab injection or rodding was done, a 180º tip was used to deliver termiticide to appropriate areas.

The statistical software used to analyze the data set was, SPSS 16.0 for windows (Chicago, Il). To compare differences between structures infested with the different species of termites at observations, a one-way analysis of variance (ANOVA) was utilized. Tukey's honest significant difference test was used to separate means.

2.2 Grid Treatment Study of 0.5% Imidacloprid Granules

The use of granular formulations of imidacloprid for the control of subterranean termites is a new concept that offers a different formulation for termite control. The granular product used in this study was ready- to- use and was transported by the pest control operators with ease. This particular product is labeled as a "kills only" product for several genera of termites including *Reticulitermes*, *Coptotermes*, *Heterotermes*, and *Zootermopsis.* It was implied by the manufacturer that the product would kill termites, but there was no claim for protection of a treated structure. Imidacloprid was advertised as a non-repellent pesticide, meaning that termites would reportedly not be able to detect the presence of the toxin, and that they would forage or tunnel into the product and "transfer" the active ingredient to nest mates via trophallaxis, grooming, and/or movement of "treated" soil which would result in the death of the colony.

To determine the effectiveness of Premise® Granules (0.5% AI) for the control of subterranean termites, a series of urban field tests were conducted at a gridded site located in Bryan, TX (GPS coordinates: 30º 37' 25. 27" N, 96º 22' 49.58" W). The field was dominated by grasses with and surrounded by predominately large Post Oak trees (*Quercus stellata*) (Fig. 3). No trees or woody undergrowth were located in any of the grids. This field was properly manicured and treated for *Solenopsis invicta* (Amdro®) prior to setup of the study. Amdro® was applied by laboratory personnel according to the manufacturer's label.

Fig. 3. Urban field setting dominated by grasses and surrounded by *Q. stellata*.

Twelve individual grids measuring 8.53 x 7.32 m (total of 62.44 square m each) were established at the study site. Corners of each grid were marked with survey flags. There was a minimum distance of 10 m between each grid. Six in-ground commercial termite monitors (Advance Termite Bait Station, BASF-*formerly Whitmire Micro-Gen,* St. Louis, MO) (Fig. 4) were evenly spaced in each grid to verify subterranean termite activity. In-ground commercial termite monitors were installed using an Ardisam Tecumseh TC II model 8900 gas powered auger (Cumberland, WI), with a15.24 cm diameter Ardisam Earth Auger Bit model # EA6F. These in-ground commercial termite monitors were installed on May 19, 2006. Each in-ground commercial termite monitor was numbered in succession starting with the 1 and ending with 72. The first inspection

of the in-ground commercial termite monitors was on June 19, 2006, at which time seven grids were found to have active subterranean termite populations in them.

Fig. 4. BASF Advance Termite Bait Station with lid and wood insert.

Six grids were randomly selected and treated with the Premise® Granules (0.5% AI) at the prescribed rate according to the manufacturer's label directions on July 24, 2006. On the morning of the treatments, six plastic containers each received 552.82 g of Premise® Granules that were weighed out on an Ainsworth model XP-1500A scale (Chicago, IL) to ensure proper weight of granules which were then applied to each of the six grids receiving Premise® Granules. The Premise® Granules were dispersed evenly with a Scotts® Handy Green II (Cinnaminson, NJ) hand held rotary spreader (setting # 4). Each of the six treated grids received 552.82 g of Premise® Granule (0.5% AI). Each grid was treated by 5 passes at 6-7 seconds each. Six additional grids served as untreated controls. After treatment, both treated and untreated each grid had six untreated southern yellow pine boards (15 x 15 x 1.5) cm placed on top of the soil and anchored with a brick. The southern yellow pine boards (surface boards) were placed a minimum of 3.05 m from the edges of the grid, a minimum of 1.22 m apart within the grid, and were 0.30 m to the right of the existing in-ground commercial termite monitors (Fig. 5). All surface boards were numbered in succession starting with 1 and ending with 72.

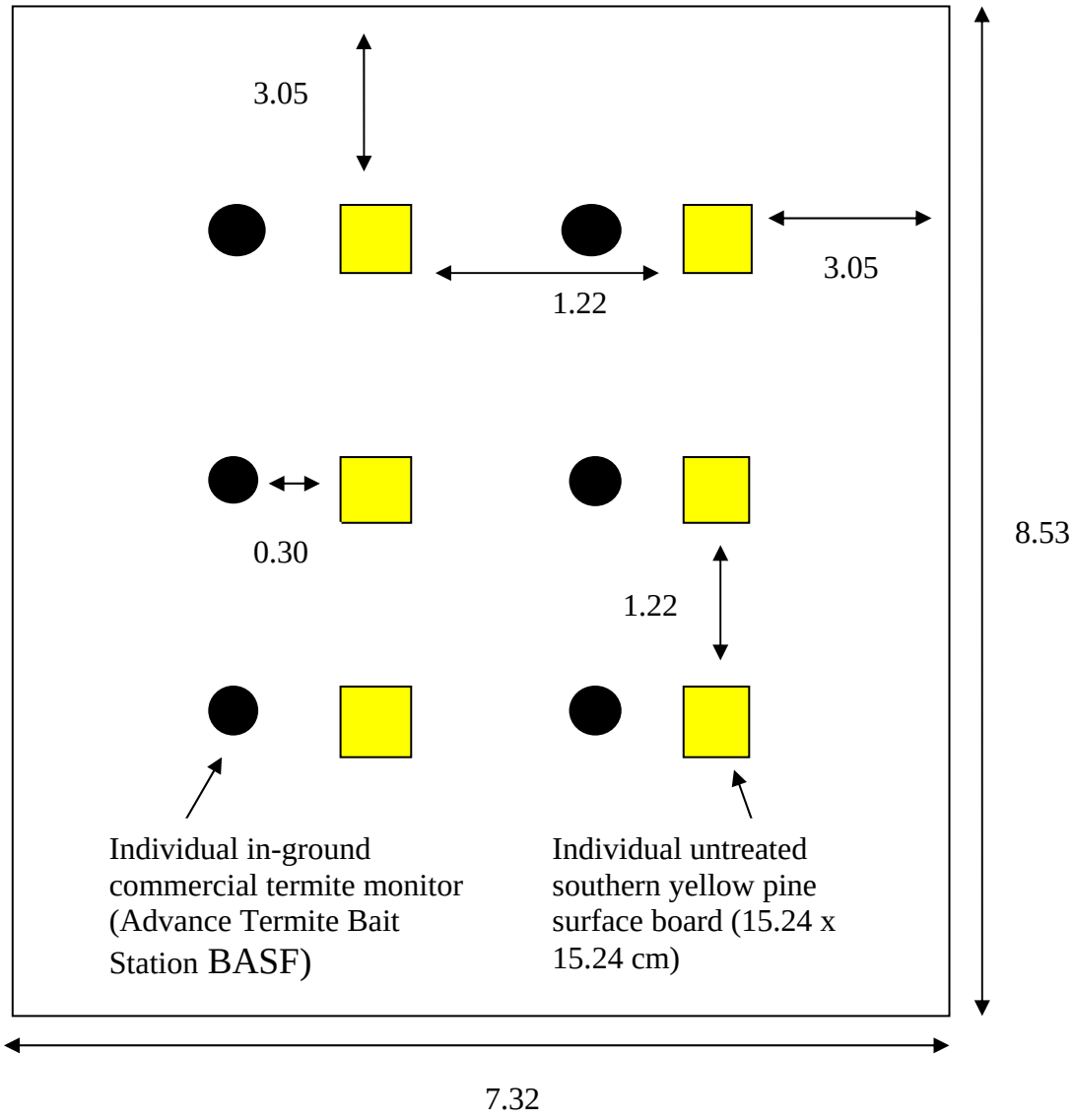

Fig. 5. Diagram of individual grid for Premise® Granule 0.5% AI study (all measurements are in meters).

Inspections of the in-ground monitors and surface boards were made at 1, 3, 6, 9, and 12 months post-treatment. Data collected were written in a National® Brand Chemistry Notebook Item #43-571 (Coppell, TX). Data collected were based on visual inspections that included the test number of the surface board/in-ground monitor and then whether or not it had been attacked by termites (termite damage but no termites present at time of inspection). If the surface board/in-ground monitor was found to be infested with termites (termites present at time of inspection), the location of each surface board/in-ground monitor (surface board #) and a rating of damage to each surface board/in-ground monitor was recorded using methods recommended by the American Society for Testing Materials (ASTM 1987, Link and De Groot 1989). Also if termites were present or damage was noted, a photo was taken of the surface board/in-ground monitor at each inspection. ASTM ratings on surface boards/in-ground monitors were cumulative throughout the duration of the study. The ASTM ratings for damage can be found in Table 1. If a surface board/in-ground-monitor insert was destroyed (rating of 0), it was replaced.

Table 1. Damage rating as prescribed by ASTM (ASTM 1987)

No. Rating	Description
10.0	No Damage
9.0	Trace Damage
7.0	Moderate Damage
4.0	Heavy Damage
0.0	Destroyed

A one-way analysis of variance (ANOVA) (SPSS 16.0 for windows Chicago, Il) was used to compare differences between treated, untreated grids, and rate of attack by subterranean termites on in-ground commercial termite monitors and surface boards placed at the study site. Tukey's honest significant difference test was used to separate means.

2.3 Structural Treatments Study of 0.5% Imidacloprid Granules

In this study initial pre-treatment inspections were done on subterranean termite-infested structures one week prior to actual treatment. During pre-treatment inspections, live termites were collected, preserved in 100% ethanol as voucher specimens, and every termite mud tube was marked and measured to a permanent benchmark (e.g., distance from corner of structure). A "spot treatment" technique was used to determine the effectiveness of Premise® Granules as a means for treating subterranean termite infestations in structures. A "spot treatment" as defined by the Texas Structural Pest Control Service in the Texas Administrative Code in Rule 7.174 as: Any treatment of a limited, defined area less than 10 linear feet (3.05 m) that is intended to protect a specific location or "spot" in which there are often times adjacent areas that are susceptible to termite infestation which are not treated (Texas Administrative Code 2009). For the purposes of this study, 10 structures (5 treatments and 5 untreated controls) with active subterranean termites on the exterior of the structure were located in a single apartment complex in Houston, TX (GPS coordinates: 29º 36' 36.07" N, 95º 13' 32.48" W). All structures were built on monolithic concrete slabs, were of the same construction type,

and were of the same age. All “spot treatments” were conducted according to the label provided by Bayer Environmental Science (Research Triangle Park, NC).

On the morning of September 22, 2006 five separate containers of 340.19 g of Premise® Granule formulation (0.5% AI) was weighed out on an Ainsworth model XP-1500A scale to ensure proper volume and weight of the treatments. At the point of infestation, a trench measuring approximately 15.24 cm wide and 15.24 cm deep was dug 1.22 m through and on either side of each active subterranean termite mud tube, which was in the center of the trench. All termite mud tubes on the treated or untreated controls were "knocked down" and scraped clean prior to treatment and at each post-treatment inspection. This was done so that, at post-treatment inspections, if a termite mud tube was re-built in the “spot treatment” area it verified that there were still active subterranean termites present. Each trench in the treatment set received 85.05 g of Premise® Granule per 0.30 m. After treatment the trench was back filled, a thin layer (1.0 g) of granules was applied to the top of the soil. There was a minimum distance of 15.24 m between all treatments and untreated controls (Kard 1998, Peterson et al. 2007). Post-treatment inspections were made at 1 wk, 2 wk, and then monthly for 12 mo post-treatment. Data collected at each inspection were visual and they were written in a National® Brand Chemistry Notebook. Data included whether or not there were active subterranean termites in the “spot treatment” zone at the time of each post-treatment inspection.

A one-way analysis of variance (ANOVA) (SPSS 16.0 for windows Chicago, Il) was used to compare differences between treated and untreated structures in this study. Tukey's honest significant difference test was used to separate means.

2.4 HPLC Analysis of Premise®75 WP from Soil and Leachate Samples

Efficacy of termiticides is a major concern to structure owners and pest control operators alike. The need for exhaustive research in this area is imperative to evaluate products used for control of subterranean termites before these products reach the market place. Areas of concern that effect the longevity of termiticides in soil include, but are not limited to, dissipation of active ingredient, uptake of active ingredient by plants, leaching of product into groundwater and/or out of target zone , and binding of the active ingredient to different soil types. All of these factors could have contributed to the degradation or the movement of the active ingredient in the environment and reduce the effectiveness of the chemical as a termiticide. This study was designed to evaluate the concentration of imidacloprid in soil over time in a series of laboratory trials that simulated an urban field setting.

In this study, 19 L buckets (Letica Corporation, Rochester, MI) had five 2.54 cm diameter holes drilled in a circular pattern in the bottom for future collection of leachate samples (Fig. 6). Fiberglass silver gray window screen (Phifer Incorporated, Tuscaloosa, AL) was cut 24 cm in diameter in a circular pattern and attached with liquid nail adhesive (Liquid Nails, Strongsville, OH) over the holes on the interior bottom of buckets (Fig. 6). The adhesive was allowed to dry for 24 hrs, after which 7.64 cm

(weighing approximately 7.14 kg) of Quikrete Premium Play Sand (Quikrete® International, Inc., Atlanta, GA) was added to the bottom of the bucket.

Fig. 6. A 19 L bucket with five 2.54 cm holes with fiberglass window screen in place to hold soil.

In order to estimate the amount of soil to be added to each experimental unit, a simulated trench was made with the following specifications, 3.05 m long by 15.24 cm wide and 15.24 cm deep (Fig. 7). This simulated trench was formed to represent a trench soil treatment next to a structure, which would follow the manufacturer's application rate of 15 L (of finished solution of termiticide) per 3.05 linear m per 0.30 m of depth. It took approximately three 19 L buckets weighing 28.19 kg each of soil to fill the form. Soil was measured to ensure proper volume of insecticide was delivered to the

desired amount of soil according to manufacturer's labeling for the use of imidacloprid to control subterranean termites.

Fig. 7. Simulated trench with the following specifications, 3.05 m long by 15.24 cm wide and 15.24 cm deep.

Three 19 L buckets of soil were added to a CF Gilco Incorporated concrete mixer (Grafton, WI) model 59020. Formulated imidacloprid (Premise® 75 WP) at the manufacturer's highest recommended application rate of 0.10% AI was slowly added to

the soil with the use of a hand-held B&G pump sprayer (B&G Equipment, Jackson, GA) using a coarse jet fan spray, as the sandy loam soil was mixed for 20 minutes at a constant rate of 20 revolutions per minute. After thorough mixing, 20.32 cm (weighing 21.05 kg) of treated soil was placed into each of the 30 19 L plastic buckets (Letica Corporation, Rochester, MI) on top of the sand that was placed in the buckets previously. Within the buckets, one of the following plants was placed to simulate structural conditions, *Stenotaphrum secundatum* (St. Augustine grass), *Cynodon dactylon* (Bermudagrass), *Cuphea hyssopifolia* (Mexican-Heather), and *Photina fraseri* (Red-tip Photinia). Bermudagrass was grown from seed (Pennington Seed, Inc., Madison, GA) purchased from a local nursery and planted in the respective buckets. Once the *C. hyssopifolia* and *P. fraseri* were planted, a thin layer of sphagnum peat moss (Miracle Gro, Marysville, OH) was applied to promote growth. There were three replications of each plant species (12 buckets), plus untreated soil controls. The untreated controls in this study included the following: no plant and no termiticide; no plant with termiticide; and, each of the four plant species with no termiticide for a total of 18 control buckets. This study was conducted over the course of one calendar year.

Soil samples were taken from the buckets at 0, 1, 3, 6, 9, and 12 months post-treatment and prepared for analysis. Soil samples were taken using a T-bar soil probe with a 25 x 2.5 cm plastic sleeve inserted in the probe to capture the soil core. After the soil sample was taken, the sleeve was properly labeled and the top received a red cap while the bottom received a blue cap. All soil samples were then placed in a freezer and held at -5°C until preparation for HPLC analysis. All sampling holes were filled with

Quikrete Premium Play Sand immediately after soil samples were taken to keep the structure of the soil in the buckets in place and so this sampling area would not be re-sampled at a later date.

In order to prepare soil samples for analysis by HPLC, the soil cores were separated into three sections: top, middle, and bottom. The top cap (red) of the sleeve was removed and approximately 15 g (8 cm) of soil (top) was placed in a 5.5 cm weighboat, properly labeled, and allowed to air dry for 24 hrs at 25±2° C. The next 15 g (8 cm) of soil was removed from the sleeve and labeled as the middle. The last 15 g (8 cm) of soil was pushed from the sleeve and was labeled as the bottom. After drying, 5 g of soil was removed from each weighboat, placed in a separate 40 ml vial, and 15 ml of acetone was added to the vial. The vial was agitated by hand for 20 seconds then allowed to sit 24 hrs. A 1 ml sample was then taken from the 40 ml vial with a micropipette and loaded in a 1.5 ml scintillation vial for analysis by HPLC. These subsamples were kept in a freezer at -5°C until analyzed. Termiticide parent residues from the soil taken through time were used to evaluate the presence of the imidacloprid parent compound concentration changes over the course of the study. The above recovery method of parent compound from soil samples was done as described by Baskaran et al. (1997and 1999).

Leachate samples (1 L) were taken at 0, 1, 3, 6, 9, and 12 months post-treatment. The plant and soil were irrigated with 2 L of water which was sufficient to fill a 1 L Nalgene bottle (Rochester, NY) with the of leachate sample. Leachate samples were taken by placing a funnel inserted in the 1 L Nalgene bottle under the bucket which was

suspended by two hollow block bricks. Preparation of 1L leachate samples was begun by prepping a Resprep™ 60 mL C18 cartridge (Restek, Bellefonte, PA) with 50 mL of 80% acetonitrile to 20% water (v:v). This was done to activate the column beads. Pressure was set so activation was within 10 minutes. A Cole Parmer vacuum pump model number L-79200-00, 115 v, 60 HZ was utilized for this process. A piece of 0.5 m long 0.31 cm ID x 0.16 cm wall tygon tubing was attached to the vacuum pump, and the other end was attached to a Resprep™ 12 port Solid Phase Extraction (SPE) Manifold. Then 150 ml of HPLC grade water were passed through the C18 columns to wash the 80:20 ACN:H_2O solution off the beads. This water was discarded as waste. Then 100 ml of leachate sample was passed through the column. After that a 120 ml Nalgene bottle was placed under the column and 100 ml of 80:20 (ACN:H_2O) was passed through the column to release the active ingredient that had bonded to the beads. The eluent was captured in 120 ml Nalgene bottles, from which 1 ml was pipetted and placed into a 1.5 ml scintillation vial. These 1.5 ml subsamples were stored at -5° C and were ready for analysis by HPLC. Leachate samples were analyzed for the concentration of imidacloprid insecticide. The above recovery method of parent compound from leachate samples was done as described by Baskaran et al. (1997). In addition to irrigation to collect leachate, routine watering was required to facilitate healthy plant growth in the test buckets.

The analysis of the leachate and soil samples was done on a 1200 series Agilent HPLC (Waldbronn, Germany). The instrument was equipped with a degasser, quaternary pump, autosampler, and an ultraviolet (UV) diode array detector (DAD).

Solvents used to extract the parent material from soil and water samples were Fischer Scientific (Fair Lawn, NJ) HPLC grade acetonitrile and water. Solvent ratio used for this analysis was 20% ACN to 80% H_2O. The injection volume was 1.0 µl, detector wave length was 270 nm with a complete analysis time of 10 minutes. The HPLC analysis performed in this study was done as described by Placke and Weber (1993), Ishii et al. (1994), Baskaran et al. (1997 and 1999), Obana et al. (2002) and Saran and Kamble (2008). Following the analysis, there was a 1 minute wash with the solvents. The column used for this process was a Zorbax Eclipse XDB-C18 analytical 4.6 x 150 mm 5-micron column.

Technical grade imidacloprid at 99.5% purity was purchased from Chem Service (West Chester, PA) to make serial dilutions. To make the stock solution for each serial dilution, 0.05 g of technical grade imidacloprid was mixed in 100 ml of acetonitrile to make a 1000 parts per million (ppm) solution. From that stock, a 10-fold dilution series was made to prepare: 0.1, 1.0, 10, 100, and 1000 ppm concentration of imidacloprid. Serial dilutions were made and utilized for each sampling period that was analyzed via HPLC. These dilutions were used to calibrate the instrument and quantify the unknown samples of imidacloprid by comparing known samples and concentrations to unknown samples. Once the serial dilutions and all unknown samples were analyzed by HPLC, the known samples (serial dilution) were plotted on a graph to construct a “best fit” line. The slope of the line and the y-intercept were calculated from the equation $y=mx+b$ along with the R^2 value for each sampling period. Retention time, height, and area of peaks were compared to quantify each unknown sample. The unknown sample values

were then inserted into the equation, and the concentration (ppm) for the samples was calculated. The serial dilutions were made at the initiation of each set of samples for each time period to calibrate the instrument. The dilutions were analyzed via HPLC with identical methods as the unquantified samples. As expected, the concentration of the diluted material was highly predictable and demonstrated little variability from the expected response throughout the study. Samples of finished solution (40 ml) were collected at time of treatment to analyze and ensure accurate mixture according to the target of 0.10% active ingredient of finished solution. These samples were analyzed with the same process as the unquantified samples.

A one-way analysis of variance (ANOVA) (SPSS 16.0 for windows Chicago, Il) was used to compare concentration of imidacloprid in plant species and observations post-treatment. Means were then separated using Tukey‘s honest significant difference test ($p = 0.05$).

3. RESULTS

3.1 Perimeter Treatment Study of Premise® 75 WSP

In setting up this study, the linear length for each structure to be treated was calculated prior to treatment to ensure that the proper volume of termiticide would be applied. The mean perimeter of all 20 structures was 66.6 ±17.8 m (Table 2). The manufacturer's label for Premise 75 WSP requires that 15 L per 3.0 linear m per 0.30 m of soil depth of finished solution be applied to the soil. The mean volume of finished solution applied per structure was 396.0 ± 164.4 L (Table 2). This number included volumes used to treat bath traps and shower pans at each structure.

Only one test structure (10%) originally infested with *R. flavipes* required re-treatment through 42 mo post-treatment study period. Active *R. flavipes* were found at structure 6 at the 9 month post-treatment inspection. The active termites were located in a base board in the kitchen. The slab entry was traced back to around the washer plumbing area. This area was treated with 0.05% AI imidacloprid. This area had not been previously treated.

Table 2. Treatment data for structures receiving a post-construction liquid application of imidacloprid (Premise® 75 WSP) for control of subterranean termites.

Structure #	Treatment group	Linear m of structure (perimeter)	Liters of Premise® applied	Liters/linear m applied
1	*Reticulitermes*	54.8	333.1	6.0
2	*Reticulitermes*	57.3	283.1	4.9
3	*Reticulitermes*	57.9	283.1	4.9
4	*Reticulitermes*	55.7	242.2	4.4
5	*Reticulitermes*	60.9	272.5	4.4
6	*Reticulitermes*	86.8	492.1	5.6
7	*Reticulitermes*	74.3	386.1	5.2
8	*Reticulitermes*	65.8	340.6	5.1
9	*Reticulitermes*	55.4	253.6	4.6
10	*Reticulitermes*	92.3	507.2	5.5
		Mean = 66.1±13.8 a	339.4±95.1 a	5.1±0.5 a
11	*Coptotermes*	45.7	227.1	4.9
12	*Coptotermes*	78.6	670.0	8.4
13	*Coptotermes*	28.0	140.0	5.0
14	*Coptotermes*	56.0	435.3	7.7
15	*Coptotermes*	64.9	325.5	5.0
16	*Coptotermes*	62.4	454.2	7.3
17	*Coptotermes*	104.8	696.5	6.6
18	*Coptotermes*	91.7	757.0	8.2
19	*Coptotermes*	67.9	393.6	5.7
20	*Coptotermes*	61.8	427.7	6.8
		Mean = 66.2±21.9 a	452.7±202.4 a	6.6±1.4 a

Linear m of structure; t=0.53, df=18, P=0.61, Liters of Premise® applied; t=1.60, df= 18, P=0.13, Liters/Linear m Applied; H=6.25, df=1,P=0.12.

Six (60%) of the ten structures infested with *C. formosanus* required re-treatments over the 42 mo post-treatment study period. The first post-treatment termite activity in this case was found during the 6 month inspection of test structures 17 and 20. At structure 20, Formosan subterranean termites swarmed out of an internal wall void near the area where one of the original pre-treatment termite mud tube tubes was found. This internal wall void had not previously been treated. This wall void was treated with imidacloprid foam at 0.05% AI. The active subterranean termites at structure 17 had re-built a mud tube on the exterior of the structure. Structure 17 was re-treated with 0.05% imidacloprid. A 9 month inspection at structure 20 was performed to follow up on the re-treatment that was performed following the 6 month inspection. Active Formosan subterranean termites were found at structure 20. This structure was re-treated for the second time.

At the 24 month post-treatment inspection, structure 14 was found to have active Formosan subterranean termites in the master bathroom which was near an area that had active termites prior to the original treatment. Structure 14 was not re-treated at that time, to allow the original treatment time to have an effect on this “new” subterranean termite infestation. At the 30 month inspection, active subterranean termites were found at structures 12, 13, 14, and 18. Of these structures 12 and 14 were treated with fipronil (0.06% AI) and were dropped from the study. Structures 13 and 18 were not re-treated at this time, to allow the original treatment to have an effect on these “new “subterranean termite infestations. In all four structures, active subterranean termites were found on the exterior of the structure, tunneling via a shelter tube on the slab. At 36 months post-

treatment structures 17 and 18 had active subterranean termites on the exterior of the structure. Structure 17 was not re-treated at that time, to allow the original treatment

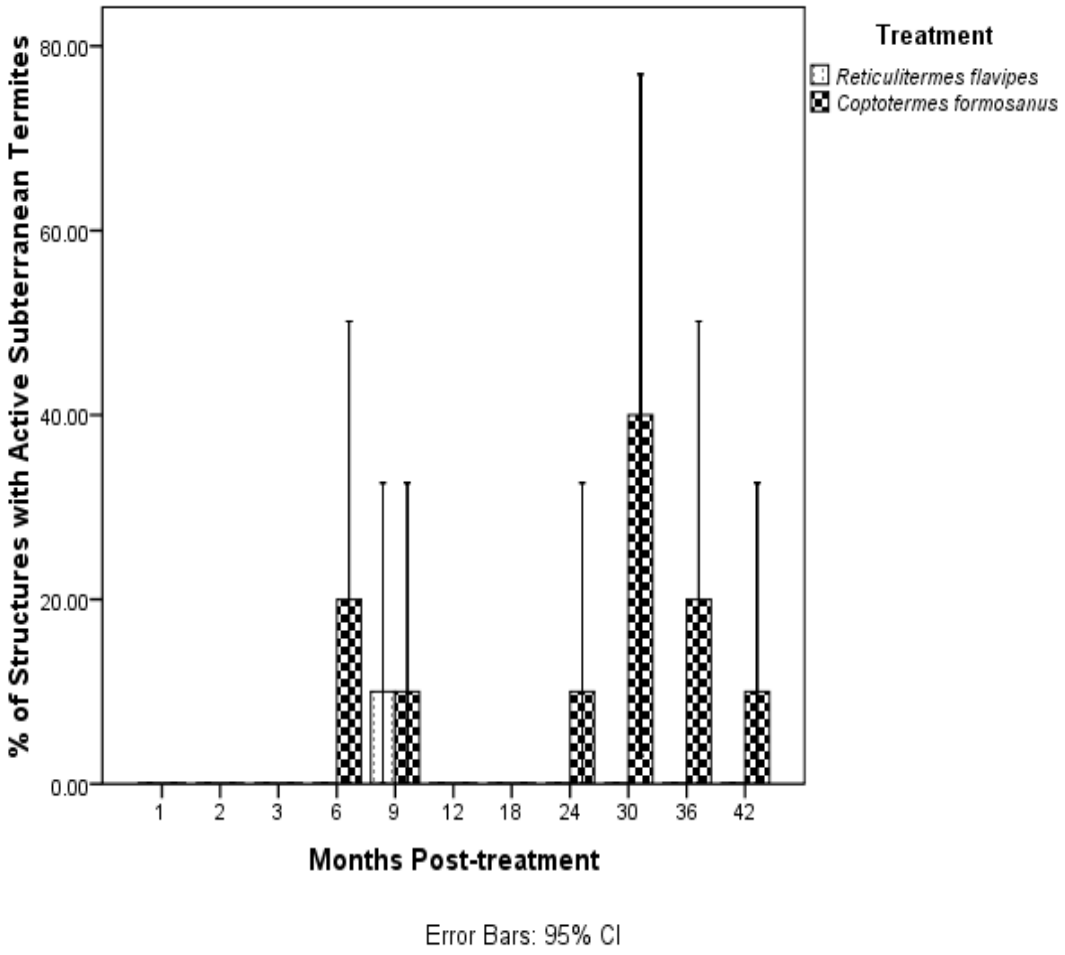

Fig. 8. Percent of structures with subterranean termite activity through time after a post-construction treatment with Premise® 75 WSP 0.05% AI.

time to have an effect, while structure 18 was re-treated with fipronil (0.06% AI) and was dropped from the study. At 42 months post-treatment there were still active subterranean termites at structure 17. Structure 17 was then treated with fipronil (0.06% AI). A complete synopsis of inspection results can be found in Figure 8.

Throughout the 42 months of inspections, six structures received re-treatments with Premise® 75 WSP 0.05% AI. In all cases, unless otherwise noted, there was no soil movement by the structure owner or animals, and there was no evidence of remodeling or other activity that would have disrupted the treatment causing a breach in the perimeter barrier.

3.2 Grid Treatment Study of 0.5% Imidacloprid Granules

After the grids were established at the test site in this study, before the grids were treated with imidacloprid granules, commercial termite monitors were installed and monitored for 1 mo. During that time subterranean termite activity was verified in 7 (58%) of the 12 grids. The following grids had confirmed activity prior to treatment with imidacloprid granules, 1, 2, 3, 4, 5, 10 and 11. Within those grids were a total of 8 monitors that had subterranean termite activity (Fig. 9).

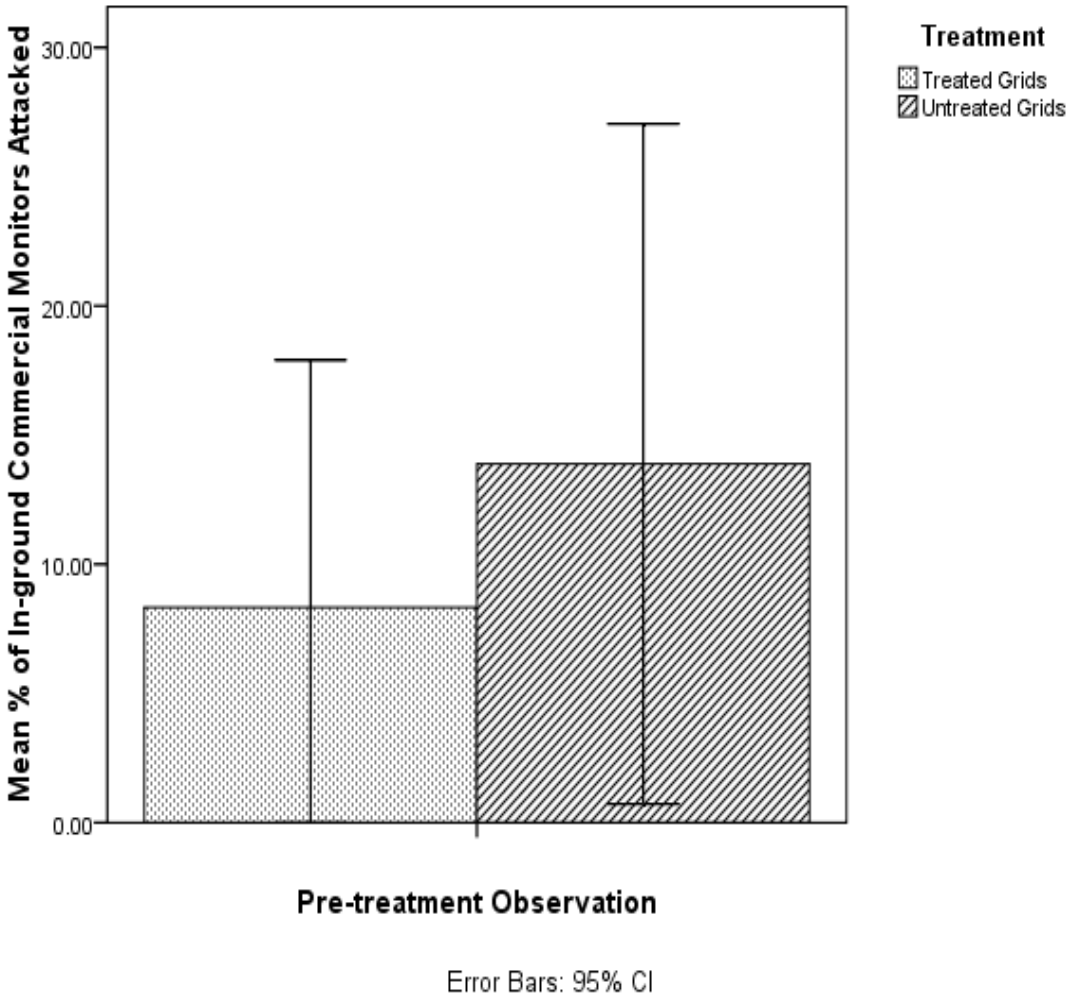

Fig. 9. Mean % of 36 in-ground commercial monitors attacked by subterranean termites prior to treatment of grids with Premise® Granules (0.5% AI)

At time of treatments, the soil was dry in the grids due to lack of rainfall. The following grids selected at random received Premise® Granules as treatments: 1, 3, 5, 7, 9, and 12. The remaining grids: (2, 4, 6, 8, 10, and 11) were sampled as untreated controls. At the 1, 3, 6, 9, and 12 mo inspections, treatment Grid 3 (treated) had active termites in at least one in-ground commercial monitor, with damage ranging from trace feeding (9.0) to heavy (4.0). In this grid, no surface boards had any activity or damage through 12 mo. At the 12 mo inspection, treatment Grid 5 (treated) had activity and damage on one monitor and two surface boards with damage ratings of moderate (7.0). Treatment Grid 7 had active termites and moderate damage in one surface board at the 12 mo inspection (Fig. 10). The mean number of monitors attacked at each inspection in the treatment grids was 2.8. The mean ASTM damage rating for those monitors was 5.7. The mean ASTM rating for the surface boards in the treatment grids was 9.4, with the only damage occurring between the 9 and 12 mo inspections. Subterranean termites were active in the untreated controls grids throughout the study; untreated control Grids 4 and 8 had subterranean termite activity at all inspection dates (Fig. 10). The mean ASTM damage ratings for surface boards and in-ground commercial termite monitors can be found in Figure 11.

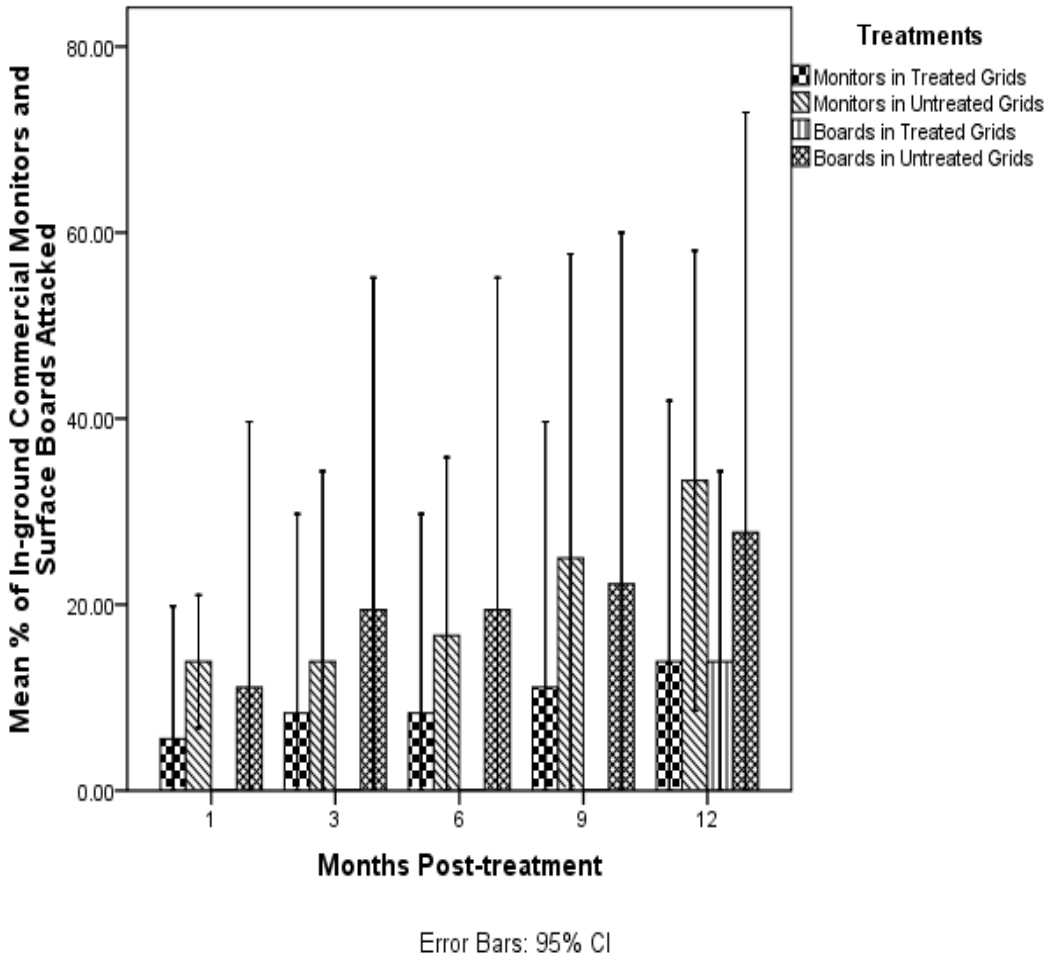

Fig. 10. Mean % of in-ground commercial monitors and surface boards attacked by *Reticulitermes flavipes* subterraean termites in grids treated with Premise® Granules 0.5% AI and in the untreated grids over post-treatment time.

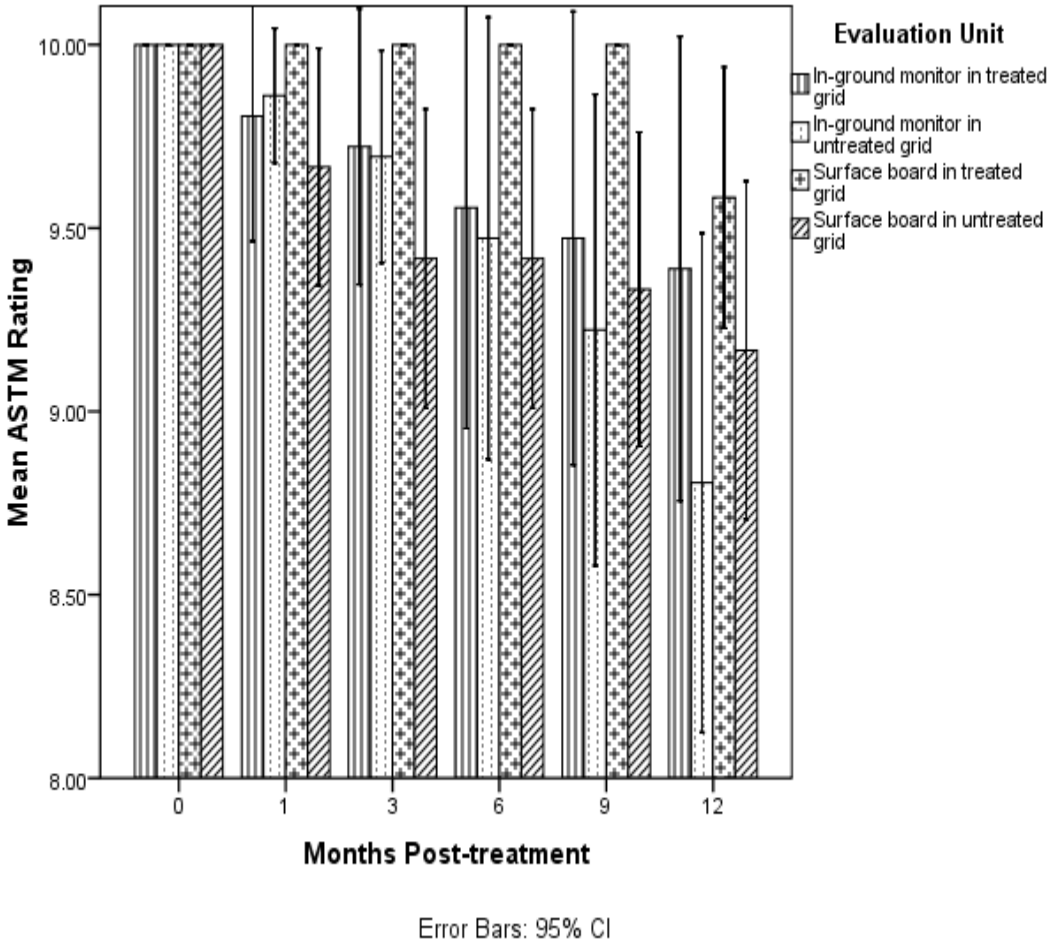

Fig. 11. Mean ASTM damage rating of in-ground monitors and surface boards attacked by subterranean termites in test and control grid used in Premise® Granule study over post-treatment period.

Total rainfall for the 12 month period was 132.38 cm, with a mean for each month of 11.02 cm. This is in contrast to the mean annual rainfall in Bryan, TX of 99.06 cm. Rainfall data were taken from Easterwood Airport, which was approximately 3.42 km south of the study site.

There was evidence of efficacy of Premise® Granules in these tests, but there were no indications that the treatments "killed" the termite colonies. This was evident

because in-ground termite monitors in the treated grids continued to be attacked by termites throughout the study. However, no damage was noted on the surface boards in the treated grids until the 12 mo inspection. The treatments appeared to suppress termite foraging just below the soil surface. This had a deleterious effect on termite feeding, which was sufficient to protect the surface boards for up to 9 mo post-treatment.

3.3 Structural Treatments Study of 0.5% Imidacloprid Granules

In this study termite mud tubes had been re-built in all five untreated control structures by the end of the first week post-treatment. These mud tubes continued to be active with subterranean termites on through the 12 mo post-treatment period. There was no activity in any of the treated structures at 1 or 2 wk post-application. At the 4 wk inspection, treatment Structure 2 had a new mud tube rebuilt 30.48 cm outside of the treatment zone (not noted as a failure). Treatment Structure 5 had a new mud tube rebuilt inside of the treatment zone, but it was inactive at the time of inspection. At the 8 wk inspection, treatment Structure 3 had an active mud tube rebuilt in the treatment zone that remained active until the end of the study. At the 8 wk inspection Treatment Structure 5, again had a new mud tube rebuilt in the treatment zone, but it was inactive. At the 12 week inspection, treatment Structure 5 had an active mud tube re-built in the treatment zone, and it remained active for the duration of the study. At the 28 week inspection, treatment Structure 1 had an active mud tube, and it remained active throughout the study. All untreated controls remained active throughout the study. By the 28 wk inspection, three (60%) of the five treated structures had subterranean termite activity within the treated zone (Fig. 12). There were no significant differences ($p=0.05$)

in the termite activity between the treatment and the untreated controls starting at wk 12 and on through the duration of the study period.

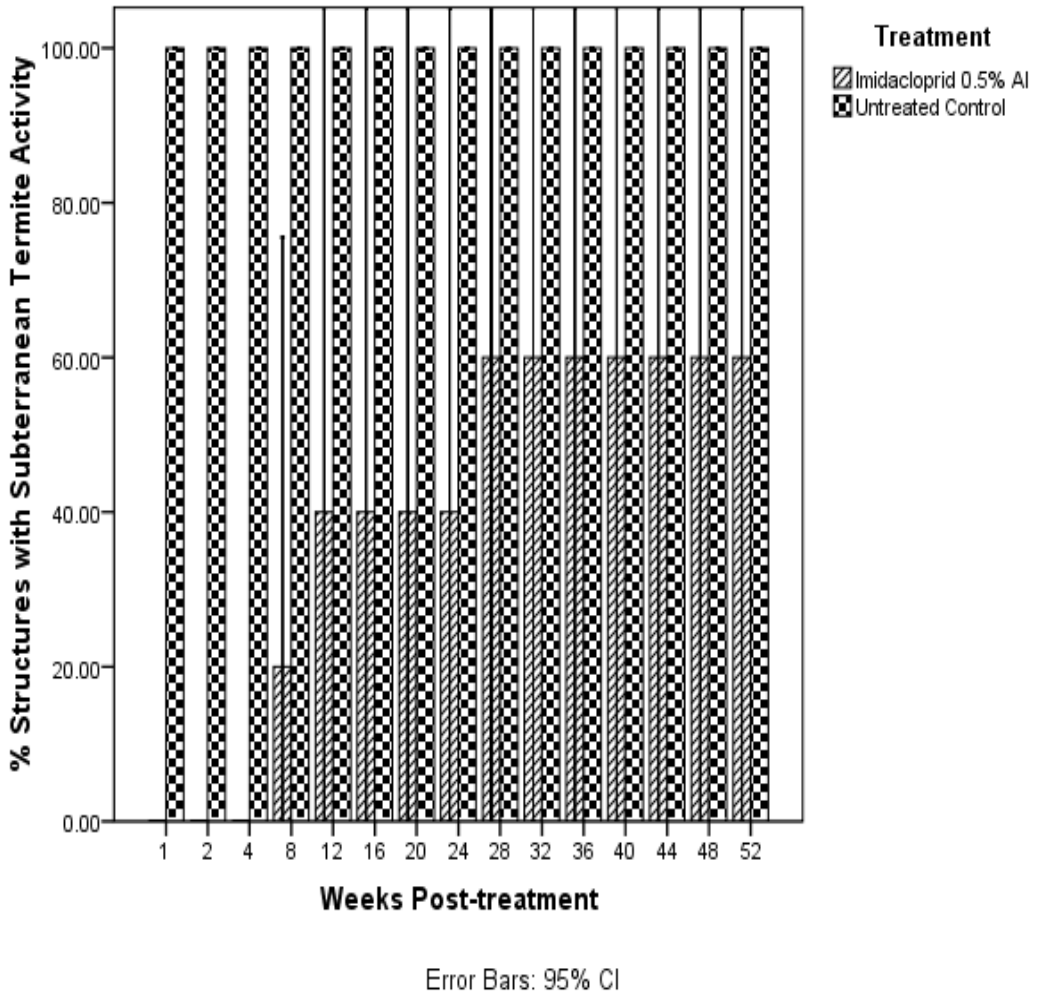

Fig. 12 Post-treatment termite activity in structures treated with Premise® Granules (Imidacloprid 0.5% AI) versus untreated structures (untreated control) over a 52 week post-treatment period.

3.4 HPLC Analysis of Premise® 75 WP from Soil and Leachate Samples

Before any samples were analyzed via HPLC, a serial dilution of known concentration imidacloprid were prepared and analyzed to calibrate the instrument. The mean retention time for the imidacloprid dilutions across all concentrations and sampling periods ranged from 4.2 to 4.3 minutes. The mean % milli-absorbance unit (mAU) followed by the standard deviation across concentrations in the serial dilutions ranged from low to high was 5.4 ±0.81(0.1 ppm), 35.8±2.4 (1.0 ppm), 148.9±26.4 (10.0 ppm), 833.8±30.41 (100.0 ppm), and 3238.9±337.8(1000.0 ppm), respectively. In this case mAU is defined as a logarithmic unit to measure optical density which had a direct relationship to the area under the peak in the chromatogram. The values that were used to calculate the above means were used to determine the ppm in the treated leachate samples taken during the current study. The mean recovery rate or yield from known concentration in ppm samples of imidacloprid in leachate was 94.2%±1.6%.

The mean retention time for the time zero leachate treatment samples was 4.3 minutes, which was uniform within 0.2 minutes throughout the study. The mean recovery concentration for the time zero imidacloprid leachate samples was 941.6 ppm (treated soil only no plants present). The mean amount of imidacloprid recovered from all plant species and the controls (no plant) at 1 mo post-treatment was 395.5 ppm. The mean amount of imidacloprid recovered at 1 mo from leachate samples by plant species including the no plant replications ranged from 301.9 to 442.6 ppm (Table 3). The 3 mo leachate samples revealed a dramatic decrease in the amount of parent material recovered from leachate samples. The mean amount of imidacloprid recovered from the

3 mo leachate samples across all plant species and the no plant replications was 21.4 ppm. The range of imidacloprid recovered from the 3 mo leachate samples from all replications was 0.0 to 66.1 ppm (Table 3). At the 6 mo sampling period there was no imidacloprid detected in any leachate sample via HPLC (Table 3).

Table 3. Mean amount of imidacloprid (Premise® WSP 75) in ppm recovered by plant species at various designated sampling periods in months post-treatment (MPT) in soil samples taken in experimental buckets through time.

MPT	Mean	Plant Species
0	941.55±10.61 a	None
1	435.17±96.91b	No Plant
	442.65±83.33b	*S. secundatum*
	409.65±108.38 bc	*C. hyssopifolia*
	387.21±85.22 bc	*C. dactylon*
	301.90±140.65 c	*P. fraseri*
3	66.12±36.02 d	NoPlant
	3.67±5.89 e	*S. secundatum*
	0.00±0.00 e	*C. hyssopifolia*
	8.09±12.04 e	*C. dactylon*
	23.31±31.43 e	*P. fraseri*
6	0.00 e	No Plant
	0.00 e	*S. secundatum*
	0.00 e	*C. hyssopifolia*
	0.00 e	*C. dactylon*
	0.00 e	*P. fraseri*

Means followed by the same letter are not significantly different ($F = 3.45$; $df = 62$; $P < 0.05$).

Before any soil samples were analyzed by HPLC a known serial dilution was analyzed to calibrate the instrument. The mean retention time for these dilution samples

in soil across all concentrations was 6.46 minutes. The mean % mAU followed by the standard deviation across concentrations in the serial dilutions ranging from low to high was 6.5±6.3 (0.1 ppm), 11.3±3.6 (1.0 ppm), 73.2±13.12 (10.0 ppm), 631.6±97.7 (100.0 ppm), and 5340.3±626.0 (1000.0 ppm). The values that were used to calculate the above means were used to determine the ppm in the unknown soil samples. The mean recovery rate from known samples of imidacloprid in soil was 84.6%±0.9%.

The mean retention time for the time zero soil treatment samples was 6.5 minutes which was uniform within 0.4 minutes throughout the study. The mean recovery concentration for the time zero soil samples was 842.6± 9.28 ppm (soil only no plants present). The mean recovery rate in 1 mo post-treatment soil samples across all soil horizons ranged from 13.90 to 22.43 ppm (Table 4). The mean amount of imidacloprid recovered from all plant species and the controls (no plant) at 1 mo was 17.7.± 16.0ppm. The mean amount of imidacloprid recovered at 1mo from soil samples by plant species including the no plant replications ranged from 13.2 to 26.1 ppm (Table 4). There were no significant differences ($p>0.05$) in the amount of imidacloprid recovered in soil samples associated with the different plant species at any time interval (Table 5). However, there were significant differences between at the 1 mo sampling period between some of the plant and no plant replications ($p<0.05$). The 3 mo soil samples had a decrease in the amount of imidacloprid recovered from soil samples which was the same trend as with the leachate samples. The mean HPLC retention time for these soil samples was 6.5 minutes. The mean amount of imidacloprid recovered from the 3 mo soil samples across all plant species and the “no plant” replications was 0.4±1.6 ppm.

The mean range of imidacloprid recovered from the 3 mo soil samples from all replications was 0.00 to 0.9 ppm (Table 5). At the 6 mo sampling period, there was no imidacloprid detected in any soil sample via HPLC (Tables 4 and 5).

Table 4. The mean concentration (ppm) of Premise® WSP 75 recovered by soil horizon through time in months post-treatment (MPT).

MPT	Soil Horizon	Mean
0	Total	842.62±9.28 a
1	Top	17.04±12.98 b
	Middle	13.90 ± 9.72 b
	Bottom	22.43±20.09 b
3	Top	0.58±1.76 c
	Middle	0.31±1.40 c
	Bottom	0.53±1.76 c
6	Top	0.00 c
	Middle	0.00 c
	Bottom	0.00 c

Means followed by the same letter were not significantly different ($F = 2.28$; $df = 92$; $P < 0.05$).

Table 5. Mean concentration (ppm) of Premise® WSP 75 recovered in soil samples associated with plant species at sampling periods through time in months post-treatment (MPT).

MPT	Plant Species	Mean
0	None	842.62±9.28 a
1	*S. secundatum*	26.65±19.53 b
	P. fraseri	20.98±8.42 b
	C. dactylon	20.09±8.54 b
	C. hyssopifolia	14.83±12.82 bc
	No plant	3.65±1.12 c
3	*S. secundatum*	0.78±1.29 d
	P. fraseri	0.59±1.13 d
	C. dactylon	0.92±1.00 d
	C. hyssopifolia	0.14±0.57 d
	No plant	0.00±0.00 d
6	*S. secundatum*	0.00 d
	P. fraseri	0.00 d
	C. dactylon	0.00 d
	C. hyssopifolia	0.00 d
	No plant	0.00 d

Means followed by the same letter were not significantly different. ($F = 4.23$; $df = 29$, $P < 0.05$).

4. DISCUSSION AND CONCLUSIONS

4.1 Perimeter Treatment Study of Premise® 75 WSP

Results of this study indicate Premise® 75 WSP provided good control of *Reticulitermes flavipes.* Only one re-treatment was necessary throughout the 42 mo of inspections on all ten of the structures included in the study. In this one incident, subterranean termites were found in the kitchen utilizing a plumbing area that had not been previously treated as a point of entry. The efficacy of Premise® 75 WSP on *Coptotermes formosanus* was more variable. There were 6 structures that received re-treatments. Some structures received more than one re-treatment. The re-treatment rate was 60% for these structures through 42 mo post-treatment. Formosan termite populations were obviously more difficult to control with Premise® 75 WSP than were *Reticulitermes flavipes* (Fig. 8). These findings support the work of Su and Scheffrahn (1990) who found that *R. flavipes* is more susceptible to termiticides than is *C. formosanus*.

Coptotermes are considered subterranean termites, but they possess the unique ability to live above ground in ideal environments. These termites build carton nests which allow them to keep an ideal range of temperature and relative humidity. This environment allows this termite to live above ground if moisture is present in a structure even when a termite treatment has been preformed. These unique abilities allow this termite to continue to live and cause damage even after structure treatment, sometimes unknowingly to pest control operators and structure owners. The most complete treatment for a *Coptotermes* infestation would be a soil treatment along with a

fumigation. However, with the advent of foam termiticides, fumigation may not always be necessary. Foam applications can be made to known areas of infestation by drilling small holes into the area and applying the foam according the manufacturer's labels.

Subterranean termites will exploit any opening through the slab and foundation of a structure to gain access to the wood framing and millwork. If liquid or granular termiticides are chosen to prevent this problem, they must be applied around the perimeter of the foundation, at any openings through the slab, cracks in the slab, and joints between abutting slabs. If termiticides are applied only around the perimeter of the foundation, the structure will not be fully protected against invasion by subterranean termites. Termiticides must be applied to and as near as possible to known infestations for maximum control of subterranean termites.

The study described herein was an accurate portrayal of events that could occur in the real world. Field studies offered a firsthand look at the problems and successes that pest control operators could anticipate in their work. Communication with structure owners and the pest control operators was critical in these field studies. Scheduling visits to inspect structures and travel to the structures, which were in some cases hundreds of miles away, was quite challenging at times. Constant contact with structure owners was necessary to establish a good working relationship which lead to less stress in communication of the results of each inspection.

Variation in structure size was large in this study. A synopsis of each treatment can be found in Table 2. The mean linear meters per structure (perimeter) was 66.6±17.8 m. The mean volume of Premise® applied at the structures was 396.0±164.4

L. The mean amount of Premise® applied per linear meter per structure was 5.8±1.2 L. These numbers are skewed due to the product that was applied in bath traps, shower pans, and wall voids.

4.2 Grid and Structural Treatment Studies of Premise® Granules 0.5% AI Imidacloprid

There was evidence of efficacy of Premise® Granules for termite control in the grid tests, but there were no indications that the treatments "killed" the termite colony. This was evident because in-ground commercial termite monitors in the treated grids continued to be attacked by termites throughout the grid treatment study (Table 4). However, no damage was noted on the surface boards in the treated grids until the 12 mo inspection. This is in contrast to results in a similar study with Premise® Granules that had damage in surface boards as early as 7 mo post-treatment (Hu et al. 2007). In the current study, the treatments did seem to suppress termite foraging just below the soil surface which had a deleterious effect on termite feeding and, which was sufficient to protect the surface boards for up to 9 mo post-treatment.

Premise® Granules are labeled as a "kills only" product. This product does not offer long term control for subterranean termites; however it does have some advantages. For example, it is a ready-to-use product, meaning there is no "mixing" of the product with water in order to make an application of the product to a structure. This is a new concept to the pest control industry, which, in the past, relied on liquid treatments that do offer long term control of termites and protected structures. Granules could be the wave of the future as far as termite control, however the author does not agree with the

practice of offering a short term treatment to a potentially long term problem with subterranean termites. Despite this concern, this practice appears to be becoming more popular as technology improves.

In the study of structures treated with granular imidacloprid, despite its non-repellency, there were several instances where subterranean termites simply moved outside the treatment zone and re-built mud tubes on a structure. Based on the results from this study, Premise® Granules again appear to offer a short term solution to the problem of subterranean termites infesting structures. Premise® Granules were effective as a post-construction treatment for remedial control of subterranean termites, but only for a period of less than one year. With that said, most structure owners are looking for longer term control when they pay for a subterranean termite treatment. With that knowledge taken into account, it was shown from the current research that Premise® Granules did not "kill" the termite colonies. This product does, however, offer some advantages to the industry including; 1) it is a ready-to-use product, and 2) it does offer some short term control. This can be an advantage, if arrangements cannot be made to offer a more conventional type subterranean termite treatment due to extenuating circumstances on the part of the client such as financial burden. In this regard, key element to the decision-making process by the client may be that a termite treatment may only be done if one of the following conditions exist: 1) evidence of live termites are present, 2) there is no evidence of a previous treatment, 3) the soil of a previous treatment has been disturbed, 4) it is proven that the concentration of a previous treatment is below the minimum inhibitory concentration, and/or 5) it has been more

than five years since the last subterranean termite treatment (M. Kelley personal communication 2010).

If the granular grid study were to be carried out again it is felt that the grids should be inspected monthly instead of quarterly. This would allow a more accurate portrayal of when the subterranean termites breached the treatments. Also it would provide a better idea of how much damage the termites could do to a structure in the time between monthly visits by a pest control operator.

In the structure treatments with granular imidacloprid, it is felt the zone of treatment should be expanded from 0.61 m either side of the active mud tube to at least 1.52 m either side of the mud tube. This would still be a "spot treatment" as defined by the Texas Department of Agriculture Structural Pest Control Service and the time required to expand the treatment zone would be minimal by a Pest Control Operator. Research needs to be performed to find the optimal treatment zone length for this product to provide better overall short term control for a structure.

4.3 HPLC Analysis of Premise® 75 WP from Soil and Leachate Samples

Recovery rates for imidacloprid in this study averaged of 84% in soil and 94% in leachate samples. The separation and detection of imidacloprid in crop-related studies has been successfully carried out with HPLC without derivatization. A reversed phase HPLC method following extraction of samples with acetonitrile/water (1/4, v/v) and cleanup using a silica gel column was developed for water and soil samples. The limits of detection of this HPLC method were between 0.005 and 0.02 mg/kg (Ishii et al. 1994). These results closely agree with those of Baskaran et al. 1997 who achieved

HPLC recovery rates of 82-88% in soil and 82-95% in leachate samples for imidacloprid.

The general environmental fate of imidacloprid has been investigated. The half-life of imidacloprid in soil is 48-190 days, depending on ground cover, organic material, and several other factors (Scholz and Spiteller 1992, Rouchard et al. 1994). The high water solubility and low organic carbon partition coefficient (K_{oc}) value seem to indicate that this compound has a low tendency to adsorb to soil particles which could play a role in this compound offering long term protection to structures against subterranean termites (Bacey 2000). The current research supports this statement as indicated by the amounts of imidacloprid recovered in leachate and soil samples taken during the course of the HPLC study (Tables 5 and 7). The soil horizon data also support this because although not significantly different ($p= 0.05$) more imidacloprid was recovered at 1 mo post-treatment in the bottom soil horizon, showing a downward movement of imidacloprid through the soil (Table 6). It would have been better, however, if there had been more imidacloprid recovered in the top soil horizon, this would represent a model whereby the active ingredient was not moving in the soil and thereby, would make the termiticide more available to the target organisms. Miles Inc. (1992) reported the half-life of imidacloprid in soils to range from 27-229 days. Other research has found that imidacloprid degraded more rapidly in the presence of vegetation as opposed to no ground cover, with estimated half-lifes of 48 and 190 days, respectively (Scholz and Spiteller 1992). In my research I found that in leachate samples associated with plants, I recovered less imidacloprid than in samples without plants (Table 5). This is in contrast

to the recovery rate of imidacloprid from soil samples in my research. In the soil samples, I recovered less imidacloprid at all time intervals in the no plant replications (Table 5). This suggests that the imidacloprid leached through the soil which is supported by my research due to the recovery rate of imidacloprid in leachate samples. Imidacloprid does show the potential to leach in runoff water, but studies conducted by Rouchard et al. (1994) and Miles Inc. (1993) showed that this compound did not reach ground water in field studies. In contrast studies, conducted in 1997 and 1998 by Bayer Corporation, it was determined that imidacloprid was capable of leaching into ground water 18 feet below the surface. The concentration of imidacloprid detected was <0.01 parts per billion to 1.0 parts per billion (Bacey 2000).

In this study, there was no imidacloprid detected after the 3 mo sampling period in both the leachate and soil samples. If the imidacloprid is not detectable, then the compound cannot be offering continued long-term protection to structures when applied as a termiticide. Imidacloprid does have some secondary metabolites which could cause deleterious effects on subterranean termites, but these were not assessed in the current study. The breakdown (hydrolysis) of imidacloprid in water results in the possibility of having several other compounds that could have deleterious effects on subterranean termites (Table 6). These secondary compounds may offer some control of subterranean termites which could lead to pro-longed effects of the treatment. This possibly needs to be further investigated.

The result of the analysis of the soil samples was similar to that of the leachate samples in this study. There was no imidacloprid detected after the 3 mo sampling

period. Again, imidacloprid could have been degraded into several metabolites in soil by various factors, and again some of these metabolites could be deleterious to subterranean termites. Insecticide efficacy in soil is influenced by many complex factors, such as mineral composition, moisture, soil temperature, soil type, pH, insecticide type, moisture, organic matter and climate (Harris 1972, Forshcler and Townsend 1996, Kamble and Saran 2005). Other key factors effecting the efficacy of termiticide are the insect's susceptibility and behavioral reaction to the chemical. The simple tolerance differences among species, life stages, castes, the mode of application, and the formulation of the compound all could play a role in the persistence of the chemical or continued effectiveness over time (Harris 1972).

Table 6. Environmental fate of imidacloprid in water and soil. (Bacey 2000)

Environment	Metabolite
Water	6-Chloro-nicotinaldehyde
	6-N-methylnicotinacidamide
	6-Chloro-3-pyridyl-methylendiamine
Soil	6-Chloronicotinic acid
	Imidacloprid guanidine
Water & Soil	Imidacloprid urea
	6-Hydroxynicotinic acid

Another key element in this study was the environment in which the study was conducted. All of the plants were housed in a secure greenhouse, which leads to elevated temperatures in the summertime. This environment also had sunlight which was available to the plants during all daylight hours. The elevated temperatures and the sunlight availability led to having to water the plants in the summer and spring seasons

to keep the plants healthy. All plants received 2L of water 3-4 times a week during the summer and 2 L of water 2-3 times a week during the spring, thus causing more leaching and chemical degradation than might be the case under field conditions.

In the structural perimeter treatment with Premise® 75 WSP at 0.05% AI study with 20 structures and two species of subterranean termites, the null hypothesis is rejected because there were differences between the re-treatment rates based on species of subterranean termites. In the case of the granule studies, Premise® Granule treatments did not suppress subterranean termites in the grid treatments, but there were no significant differences between the treated and untreated grids based on data. In this study, the null hypothesis is accepted; i.e:, that there is no significant difference between grids treated with Premise® Granules and untreated controls. In the structural treatments with Premise® Granules there were significant differences ($p=0.05$) up to the 12 wk inspection period. At the 12 wk inspection there were no significant differences between the untreated controls and the treatments, and this remained so for the duration of the study (Fig. 11). In this study the null hypothesis is rejected because there were significant differences between the structures treated with Premise® Granules and the untreated controls. In the HPLC laboratory study there were differences between the amount of imidacloprid recovered at different time periods between the treatments with plants and the control (no plant). In this study, the null hypothesis that there would be no differences between treatments and controls is rejected.

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