Final Report[1]

Research Proposal to the California Structural Pest Control Board for Structural Pest Control Research #084-3635-6

Assessment of Devices and Techniques for Improving Inspection and

Evaluation of Treatments for Inaccessible Drywood Termite Infestations

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[1] The statements, figures, and tables contained in this report fulfill the reporting requirements of the contract and have not undergone peer review. Copies of all final peer-reviewed and accepted manuscripts from this report will be forwarded to the Structural Pest Control Board at a later date.

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Executive Summary

Laboratory and field studies were conducted to evaluate and/or improve technology for the inspection and treatment of inaccessible drywood termite infestations. Our focus was to evaluate the usefulness of X-ray and temperature-enhanced infrared technologies in finding drywood termite infestations hidden behind wall coverings, such as drywall, stucco, and wood paneling, in both laboratory and field settings. These technologies exploit changes in wood density and temperature-response characteristics caused by the damaging effects of termites. Verification of active infestations of drywood termites included the use of commercially available microwave and acoustic emission (AE) devices. Field evaluations of these technologies were conducted in partnership with local pest control operators. Critical to these studies was documentation of (1) the constitution a drywood termite colony and (2) the frequency of feeding. Currently, the definition of a drywood termite colony is based on location; infested boards that are widely separated are assumed to contain different colonies. There are several biological definitions of what constitutes a drywood termite colony for conditions in California. The genetics of workers and chemical analyses of fecal pellets were used to separate colonies of drywood termites and to provide a measure of successful or failed treatments. Lastly, we found that drywood termites have uneven feeding patterns throughout a day and during the year. We used a computerized, multi-channel AE recording device to document variation in drywood termite feeding. The combination of these studies will allow for the better calibration of detection devices that exploit termite feeding and motion.

Background

Economic importance. Surveys of inspection reports reveal that drywood termites have a significant economic impact in California. One of the eight species of drywood termites found in California, *Incisitermes minor* (Hagen), has the greatest economic importance. Visual evidence of drywood termites contained in the wood-destroying pest inspection reports suggests drywood termite infestation rates can be as high as 46.8% of the structures inspected. Control of drywood termite infestations is predominantly by applying local treatments, not whole structure fumigations. Costs for control and repair of damage from drywood termites in California exceeds $300 million annually and are projected to increase. Not included in these costs are fears and perceived risks from treatments such as fumigation.

Visual inspections. A flashlight and metal probe are used to search for evidence of drywood termites: alate wings, fecal pellets, and damage to wood. The effectiveness of visual searches is unknown. Characteristic fecal pellets are considered diagnostic for drywood termites. The presence of fecal pellets has been used to demonstrate efficacy of chemical treatments, however there are no published reports on the chemical nature of pellets that relate to the active status of a drywood termite infestation.

Inaccessible infestations. The greatest challenged faced by PCOs during their inspections is to identify the existence and extent of inaccessible drywood termite infestations. For most filed reports, areas not inspected are noted as inaccessible to visual search. Without removing wall coverings, it is impossible to reliably delimit infestations behind walls. From my personal experience in southern California inspecting apartments for dyrwood termite infestations, roughly one infested board was found for every 10

boards exposed after wall removal of covering. Because over 70% of treatments for drywood termites in California are localized, there is a critical need to be able to locate and circumscribe active, inaccessible infestations of drywood termites.

Alternatives to visual searches. There are at least seven devices and methods proposed as detection alternatives to visual searches. They include optical borescopes, dogs, electronic odor detectors, acoustic emission devices, microwaves, infrared, and X-ray. They all claim high levels of successful detection of termites; however, few have been scientifically tested.

Optical borescopes currently marketed use visual light passing through a hollow tube as a means to view evidence of drywood termites and damage within wall voids. A small hole must be drilled into walls to allow viewing. Fire blocking, insulation, and viewing through a fish-eye lens can impede the inspector's view.

Dogs have also been used to assist with drywood termite inspections, although scientific studies verifying the effectiveness are scarce. Dogs find termites by hearing or smell or both. In California, the effectiveness of beagles in finding subterranean termites infestations has been mixed. There have been no investigations in California to assess the ability of dogs to locate drywood termite infestations. Laboratory trials conducted in Florida found that dogs (beagle and German shepherd) correctly identified drywood termites in plastic containers 88.8% of the time. Currently, there are few commercial firms in the USA that train and provide dogs to assist with termite inspections.

Electronic odor detectors detect methane gas, commonly produced by termites. One device (Termitect II) was tested on subterranean termites and produce highly variable detection rates, 20 to 100%. There have been no reports on the use of electronic odor detectors successfully locating drywood termites, even though they produce methane.

Termites produce vibrations in wood; some can be heard by humans. Sounds made by drywood termites are produced during feeding and by vibratory movements of workers. The earliest commercial listening device for termites was the INSECTA-SCOPE. No data are available on its performance. Newer technology, that amplifies and records termite-feeding vibrations, is acoustic emission (AE). Surface and subsurface probes are available that successfully detect drywood termites in laboratory settings. Wall covering can impede sensor and AE performance. Detection is limited to ≈80 cm along the length of a board and < 8 cm across the grain. Excessive background noise can result in falsely identifying active drywood termite infestations. AE detection equipment is commercially available, although availability is very limited.

Microwaves comprise the frequency range 0.3 to 300Ghz and lie between radio and infrared region of the electromagnetic spectrum. Microwaves are commonplace in our daily lives and their commercial applications are many and varied. The use of microwaves as a commercial method for localized drywood termite control has been available in California for at least 15 years. Recently, portable microwave detection devices have been marketed in California. Success in detection of drywood termites using microwaves (TERM_A_TRAC™) was 86% in Australian laboratory studies. Detection distance was 35 mm along the long axis of test boards and 25 mm deep below the surface. However, water in wood, wall coverings, and excessive wind and motion can lead to false positives.

Infrared rays are part of the electromagnetic spectrum. Although invisible to the human eye, infrared energy has a penetrating heating effect. Most objects, living or not,

give off infrared heat, whether internally generated or reflected. Initial uses of infrared were for military surveillance. Today, there are many nonmilitary uses and include measuring devices, binoculars, and night viewing for hunting. In structures, infrared devices have been used to find faulty electrical connections and heat and water leaks in walls and roofs; a more recent use includes termite detection. Commercial termite detection models are available, yet their effectiveness in finding termite infestations has not been scientifically tested. Infrared imaging has been used to detect internal voids intended to represent voids generated from biodeterioration. The results of these tests were promising, although detecting subsurface defects (voids) clearly represents a challenge.

Penetrating rays are part of the electromagnetic spectrum nearest ultraviolet rays. They are invisible, have smaller wavelength, and have a higher frequency (Hz) compared to the visible part of the spectrum. There are many commercial applications for X-rays. X-rays have the ability to penetrate nearly all materials. These penetrating rays have also been used to nondestructively view insects hidden in wood for at least seven decades. X-rays have been used to view structure-infesting beetle pests. Only recently has the potential use of X-ray in detecting drywood termite infestations been explored. Today's newer technology is lightweight and portable. Processing of images is accomplished with lasers that produce digital images that are enhanced by computer software. Newer sources of X-rays emitters and advances in safety monitoring equipment minimize radiation leakage and exposure.

Seasonal activity and pellets as indicators of failed treatments. Seasonal activity patterns of drywood termites are very important for their detection and treatment. A common seasonally activity for drywood termites is swarming. In California, drywood termites annually swarm starting in summer continuing into fall. Little is known about when drywood termite feeding occurs. A commonly held belief is that drywood termites feed every hour of every day. The cryptic nature of drywood termites deep inside wood hinders studies that explore their normal feeding behavior. Early laboratory studies using AE technology to record drywood termite feeding found no periodicity in feeding in a 24-hour day. An unpublished field investigation in southern California, using AE monitoring of local chemical treatments, found drywood termite infestations in untreated locations of structures declined in activity during winter months. The movement of drywood termite may also be seasonally based, however the seasonality of movement for drywood termites within structures in California remains poorly understood. We need to determine whether drywood termite feeding and movement are seasonally based. These results will have a profound affect on inspections, and post-treatment evaluations for failures of localized treatments.

As drywood termite feed they void the undigested components as very dry fecal pellets. These pellets are hexagonal in cross section and are diagnostic for drywood termites. The number of pellets produced per termite is about one per day. We do not know whether changes in the chemical nature of pellets voided by drywood termites can indicate the active or in-active status of infestations.

Cuticular hydrocarbons are long-chain carbon molecules and are part of the waterproofing mixture of waxes on the outside surface of termites. These characteristic mixtures can be used to identify termite species and have been reported for all castes of termites. However, the most conspicuous evidence of drywood termite infestations,

voided fecal pellets and the chemicals they contain, has only recently been researched. Pellets of drywood termites contain the same mixture of hydrocarbons as the insects that produce them, in reasonably equivalent proportions. A need exists to explore chemicals contained in pellets that might indicate whether an infestation is active or inactive.

Molecular genetics. The use of genetic markers offers a powerful way to investigate colony social organization, and there has been a growing number of genetic studies of social organization in termites. Microsatellite genetic markers are especially useful markers for studies of colony and population genetic structure and for tracking the fate of individual colonies. There have been microsatellite markers developed for an increasing number of termite species, but until recently no microsatellites have been developed for any drywood termite species. Development of microsatellite markers for drywood termites will provide three new, practical techniques. First, a biological determination of what constitutes a colony of drywood termites will be established. Secondly, once the members of a colony have a genetic identity, failed treatments can now be determined if a new or different colony is found in the treated structure. Lastly, once it is firmly established what a drywood termite colony is, new least toxic treatment strategies, for example baits, can be developed and tested.

Research Objectives

The objectives of the research plan were ambitious with multiple components to identify and delimit inaccessible drywood termite infestations. Novel devices that included X-ray, infrared, AE, fiber optics (borescope), and microwaves were used to demonstrate that drywood termite infestations can be located nondestructively and accurately mapped out during the inspection process prior to treatment. The biological meaning of a drywood termite colony was investigated. Genetic and chemical techniques provide analytical baseline data that allow for the labeling of drywood termite colonies as being active, inactive, as well as treatment failures. The results of each research objective are summarized below.

1) Determine the effectiveness of the combination of X-ray, temperature enhanced infrared, AE, borescope, and microwaves in finding inaccessible drywood termite infestations in laboratory boards.

Laboratory and field investigations were conducted on termite detection technologies that included fiber optics (borescope), Acoustic emissions (AE), infrared, and X-ray. For laboratory investigations, methods were developed for bench-top testing for the borescope. Additional methods were designed to calibrate X-ray and infrared camera for viewing artificially and naturally infested boards containing drywood termites. For the borescope, seven items were presented to seven laboratory participants in 5.5 cm clear plastic, sealed dishes. The design included the added features of being able to differentiate ants from termites, drywood termite fecal pellets from plant seeds, and pellets from debris. The ability to identify untreated checks (empty plastic zipper cases) was also included in the design. All participants had a high level of successfully identifying items presented them in plastic, sealed dishes, at least 90%. Similarly the success rate in correctly identifying untreated checks among participants was also high, 100%. Laboratory investigations produced procedures and techniques to attempt field

investigations involving infrared and X-ray that included exposure time and post-processing and enhancement of images obtained.

2) Determine the effectiveness of the combination of X-ray, temperature enhanced infrared, AE, fiber optics (borescope), and microwaves in finding inaccessible drywood termite infestations under field conditions.

For field investigations we used the Villa Termiti, a 400 ft^2 (37.2 m^2) wooden structure designed and constructed to compare termite detection and treatment technology. The testing protocol included the use of test boards that were active for drywood termites, inactive (damage only), as well as untreated checks (controls). In total, nine boards were searched with each microwave, AE, infrared, and X-ray equipment. The objective for these tests was to determine if any of these devices could singularly identify the presence of damage and active termites in test boards. An additional challenge or variable was to determine capability of these devices to make detection determinations through wall coverings that include wood paneling, dry wall, and stucco. At the end of field investigations, all test boards were cut into 10 cm sections and dissected for live and dead termites. Notes were also taken for the presence and location of gallery architecture. Under the field conditions of the Villa Termiti, only X-ray demonstrated the ability to accurately detect the presence of damage and termites, albeit these results were best for exposed boards. We were unable to determine whether boards were active with either termites or damage using infrared camera, even after heating the boards for 10 sec at 540^o C. AE and microwave produced reliable results (all test boards determined to be active with termites using both these techniques were verified active upon dissection), but were impeded when wall coverings were added, due to thickness or difficulty in drilling sensor-testing holes. Results using a borescope in blind tests presented to seven participants were mixed and highly variable depending on the number of pellets, presence of installation, as well as the nimbleness and the ability among participants to bend down low to manipulate small drilled holes and tight wall void space. For infrared and X-ray, several hundred images of laboratory boards containing termites and actual real-world field conditions have been created and stored in a photo solon.

At this time we cannot recommend a single detection device that can reliably, with a high degree of certainty, demonstrate that termites are active in walls, especially for high locations (vaulted ceilings) and when wall coverings are in place. No, there is not a single “silver bullet.” The best option for drywood termite, or any other wood destroying insect, inspection is a trained operator with 20 years of field experience and permission to open walls for further inspection. The next best option would be after identifying a wall or better yet a single board, use an AE device with a subsurface sensor (small wood screw sensor) to determine the board is active for drywood termites. A second device, this time a portable microwave sensor, can be passed held against the board or even drywall (be careful not to hold the device, best to use a tripod to minimize false positives due to vibrations) to aid in identifying the exact locations of galleries. This detection strategy works best for local treatment, where the exact knowledge of active boards and gallery architecture is critical to success.

3) Determine the nature and limits of drywood termite colonies in laboratory maintained boards and actual field infestations using molecular genetic markers.

Given the vast economic impact of western drywood termite, the understanding of population genetic structure and breeding systems is fundamental to the development of more effective management strategies. Modern molecular markers, such as microsatellites, are now commonly used to address critical biological questions. Microsatellite DNA consists of tandem repeats of between 1 and 6 base pairs, often in long arrays, and can comprise a large fraction of a termite's genome. Microsatellite markers offer the greatest potential for studies relating to breeding structure and colony identification, due in part to their co-dominant, Mendelian mode of inheritance, high mutation rate, high level of polymorphism, and ability to be amplified from small amounts of tissue. Microsatellites do not contribute to the genetic code for protein synthesis, thus there is no selection pressure, and the highly mutated sequences are inherited and passed on within a colony.

We developed an enriched library from which we isolated and characterized 15 polymorphic markers. The number of unique alleles per microsatellite locus ranged from three to fifteen. These markers yielded sufficient within and between colony/population polymorphism for the resolution of patterns of gene flow and colony breeding structure.

Termite colonies are considered to fall into three breeding categories. When reproduction involves only a single king and queen, the colony is referred to as a simple family. Following the death of one or both primary reproductives, the colony may undergo several rounds of inbreeding as a result of reproduction by secondary neotenics. This is referred to as an extended family. Finally, colonies have been shown to fuse: workers within a colony exhibit genetic ancestry to more than one colony. These are referred to as fused or mixed family colonies.

The objective of this study was to determine the levels of genetic diversity within geographically separate colonies of *I. minor* using a set of species specific microsatellite markers. Within two landscapes, urban and agricultural, we used these markers to examine the colony genetic structure, identity, and breeding structure. A total of 23 colonies were sampled from 11 distinct collection sites in California, 10 sites were classified as urban. Genomic DNA was extracted from 10 to 20 workers termites per site. These individuals were genotyped at five polymorphic microsatellite loci. Colonies were placed into one of three breeding structure groups: simple family, extended family, and mixed family colonies. Colony genetic structure was performed over all samples, among urban samples only, and among agricultural samples only.

Eleven of the 13 collections were considered unique colonies. Of the 13 urban samples, six colonies yielded genotypes consistent with those expected under a single pair of reproductives. No more than four genotypic classes were detected, all segregating with Mendelian ratios expected for a single pair of reproductives. Three colonies, while possessing no more than four alleles per locus, exhibited greater than four genotypic classes (i.e. too many homozygous genotype classes), or Mendelian segregation patterns inconsistent with a single pair of reproductives. These were therefore determined to be extended families. The remaining four colonies exhibited greater than 4 alleles at one or more loci thus providing evidence for colony fusion or mixed colony structure.

All colonies sampled within the agricultural landscape were genetically distinct. Within the seven agricultural collections three colonies meet the criteria of being simple families. A further three exhibited an excess of genotypic classes while possessing no more than four alleles at each locus, and were therefore identified as extended families. The final colony exhibited greater than 4 alleles at more than one locus and was determined to be a mixed family.

Results presented here represent the first documentation of breeding structure, colony identification, and colony genetic structure to date for *I. minor* using highly polymorphic molecular markers. The high level of polymorphism (12 to 32 alleles per locus) exhibited at these five loci proved highly suitable for colony identification, and therefore may represent a powerful tool for future studies investigating colony survival and re-infestation events post insecticide treatment, informing the pest management professional of the efficiency of a given treatment.

Colonies composed of a single pair of reproductives and their worker/soldier progeny probably represent colonies that are up to or just over 5 years of age. These are colonies that have yet to produce neotenic reproductives or those that contain neotenics that have not yet produced progeny. Extended families composed of multiple reproductive neotenics represent colonies that are probably 10 years of age or older, and may be responsible for extensive structural damage to a building. Mixed family colonies, from colony fusion, may prove difficult to explain. Explanations as to how these mixed family colonies form have been broadly divided into two categories: those driven by worker foraging behaviors and those driven my alate reproductive strategies. Thus in this instance colonies may not actually represent a mixing of genetic lineages within a large colony, but rather an event were colony foraging areas overlap. An alternative explanation may be that samples collected that appear genetically as being fused/mixed may actually represent distinct colonies whose activity centers or nests were proximate within the sampled wood, but not actually fused. Thus, two distinct colonies may have been inadvertently collected and labeled as a single colony.

Given the significant economic impact of *I. minor* in the western U.S., the ability to accurately identify colonies, their breeding structure and genetic structure is of fundamental importance for the formulation of effective management strategies. We present the first results generated using highly informative microsatellite markers that will allow the following to be estimated with relative ease:

1) Colony size and age. Simple family structure may inform us that colonies are relatively small, detected in the early stages of infestation. Higher levels of inbreeding and the determination of colonies as being extended informs us that the colonies are likely to be extensive, containing numerous secondary reproductives each producing workers. Thus given the cryptic nature of this species we can now make an informed decision as to the likely extent of damage without extensive physical investigations of a structure.

2) Colony range. The treatment method employed may vary significantly depending on its range. Using these markers we can determine whether colonies are contained to small regions of a property, or if they extend throughout a building. Thus, in instances of a single colony ranging throughout a structure, less environmentally intensive insecticide treatment (for example baits) may be developed to transmitted through the nest system from a single treatment point.

3) Treatment efficacy. When a structure is treated, resulting in apparent colony extinction, its efficacy can be determined through genetic testing. Sampling individuals prior to, and after, the treatment, assuming a structure becomes reinfested, will allow us to determine whether the original colony was successfully exterminated and therefore determine whether re-infestation occurred from an outside source, or if a fragment of the original colony survived to re-infest the structure.

4) Explore the existence of qualitative and quantitative differences in chemicals in fecal pellets, using cuticular hydrocarbon analyses, that lend themselves to determining the active or inactive status of drywood termite infestations.

Remedial control of drywood termites in the US relies primarily on (1) fumigation of the entire structure or (2) localized chemical or physical treatments designed to eradicate localized colonies. The presence of drywood termites is usually determined by the appearance of fecal pellets, which are ejected through a "kick hole" in the external surface of wood.

When fecal pellets appear after remedial treatment of a structure, it is difficult to determine whether the termites in the structure are alive or dead. Conveniently these fecal pellets contain the same mixture of hydrocarbons as the cuticle of the insects that produced them. The hydrocarbons in the fecal pellets can even be used to identify the termite species.

Rather than simply signaling the general presence of termites or providing a precise diagnosis of the species of termite inhabiting the wood, we wondered whether these pellets could be chemically characterized to determine the active or inactive status of a colony. We quantified the hydrocarbons in pellets of *I. minor* aged for up to one year after they were produced. We documented the changes in proportions of selected hydrocarbons as an indication of the age of the colony producing them.

Drywood termites were removed from a naturally infested board and placed on birch tongue depressors that were bundled together, placed in a plastic container, then lightly misted with water. Termites were held in a dark cabinet under ambient laboratory conditions prior to collection of fecal pellets. At the end of one week a 200 mg sample of fecal pellets was divided into three replicates each of four aging intervals: 1 week, 1 month, 3 months, and 1 year from the initial collection date. The hydrocarbons from fecal pellets were extracted, characterized, and quantified at the end of each time period.

The percentage of the total hydrocarbon for each hydrocarbon peak for each aging period was the response of interest. These percentages were regressed against the days of aging. Hydrocarbons with slopes statistically different from 0 were separated into two groups, those with a positive slope and those with a negative slope. For each aging period, an index of age (I_{age}) was created by subtracting the sum of the percentages of the hydrocarbons with a significant negative slope from the sum of the percentages of the hydrocarbons with a significant positive slope [$I_{age} = \sum (PEAK_{positive}) - \sum (PEAK_{negative})$]. The set of twelve I_{age} values (3 replications times 4 ages) was then regressed against age.

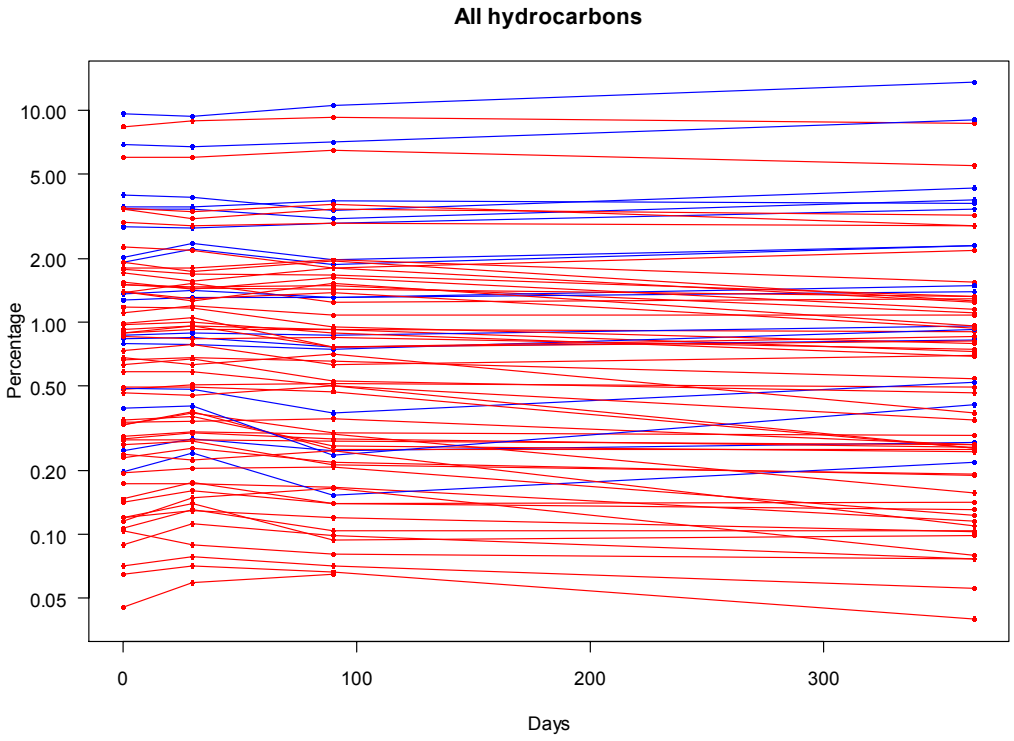
We identified 72 peaks containing 76 hydrocarbons. The change in relative abundance for each hydrocarbon peak over the course of a year does not appear, at first glance, to be very dramatic. However, 19 of the 72 hydrocarbon peaks (26%) had a

significant linear change over time; of those 5 had positive slopes and 14 had negative slopes.

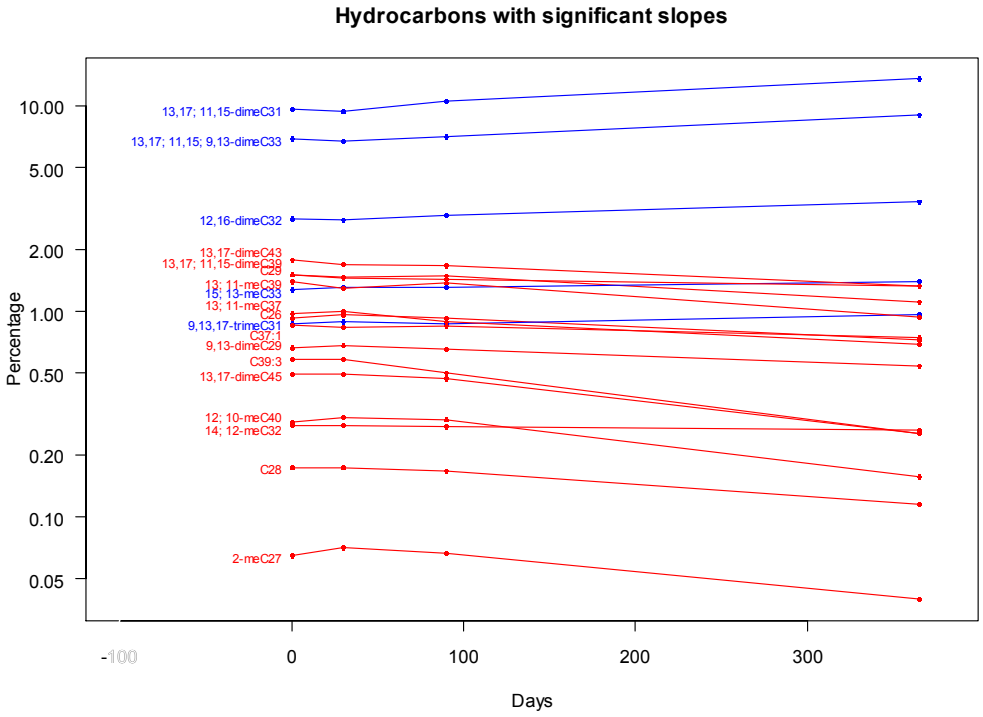
We took the 19 hydrocarbon peaks with significant regressions and created an index, I_{age}. A line was fit ($r^2 = 0.8879$) to show the variability of the replicates about the fitted line and the trend over time. This highly significant correlation strongly suggests that one might be able to predict the age of fecal pellets and thus the status of the colony that produced them.

At this point the chemical mechanism by which hydrocarbons increase or decrease in proportion over a one-year period is unknown. It is not clear to us that the hydrocarbons that increase in relative abundance are inherently more stable than those that decrease in relative abundance. Those that increased in relative abundance tended to have higher relative abundances to begin with.

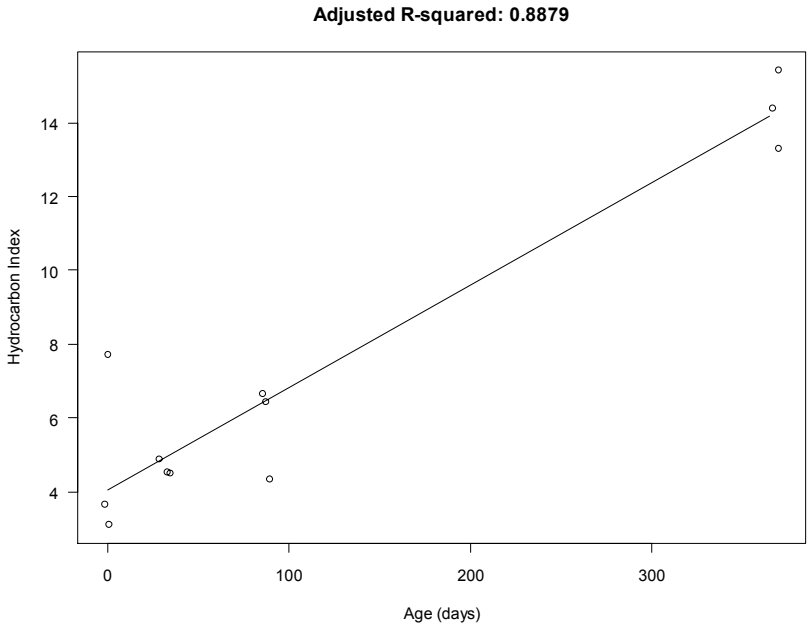
The motivation for this work was to aid in the post-treatment evaluation of success or failure for drywood termite treatments. We felt that there would be considerable interest by the industry, regulatory agencies, and consumers for a simple and accurate means to determine how long a "colony/infestation" has been in a structure.



Plots of the log of all hydrocarbon percentages of fecal pellets over time (averaged over the three replicates). A log scaling of the percentages is used to allow more visual separation of the individual hydrocarbon histories.



Histories of fecal pellet hydrocarbons with significant slopes over time.



Plot of index of age (I_{age}) against age.

5) Determine the seasonality in drywood termite foraging and feeding using AE remote sensing equipment.

Daily and seasonal activity patterns of drywood termites, such as feeding, are very important for the detection and treatment of drywood termite infestations. The cryptic behavior of drywood termites deep inside wood hinders studies to directly observe their behavior. The purpose of this objective was to explore diurnal and seasonal patterns of drywood termite feeding activity in naturally infested wood.

Logs from a large Loquat tree were collected from a private residence in southern California. The logs were similar in diameter, length, and age. To verify that candidate logs contained an active drywood termite population, we took three 1-minute AE recordings using a hand-held device. Seven logs producing at least 300 AE counts per minute were included in the study. Five of the seven logs were randomly chosen to record AE activity of drywood termites. Two of the seven logs were treated to kill all of the termites were used to measure background AE activity from the surroundings.

All seven logs had a subsurface sensor installed into their center, 1.2 cm deep into wood. A 3-meter-long cable from each of the seven sensors was connected into a port in the back of an AE smart device and a dedicated computer that stored all data. Twenty, 3-minute recordings were recorded randomly among the seven sensors for each 60-minute period during the study. In addition, temperature and humidity data were recorded for each 3-minute recording. The entire AE gathering and storage system was run 24 hours a day for 11 months (June 2008 to May 2009).

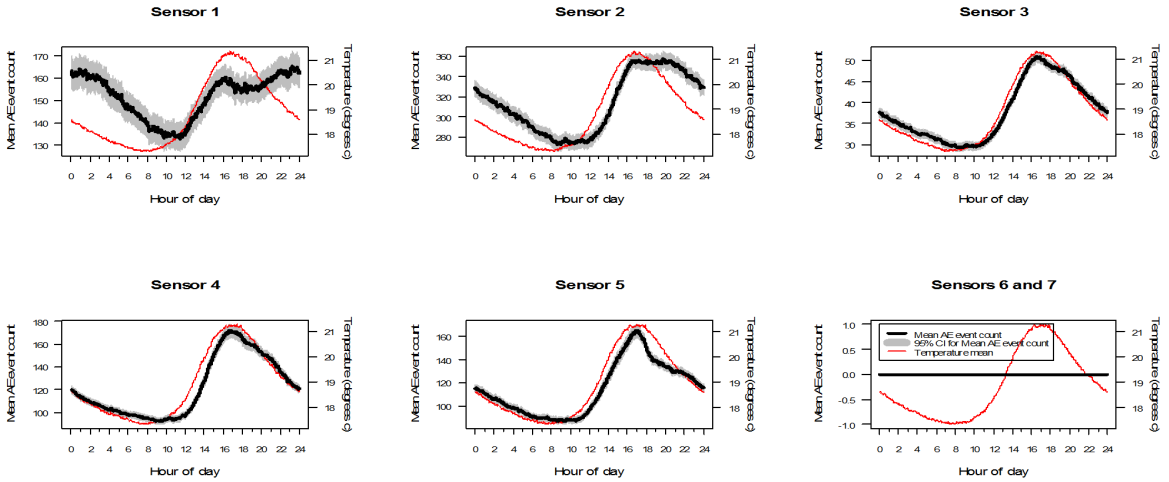
All logs, AE and temperature equipment were stored in a small wooden building at the University of California Richmond Field Station in Richmond, CA. The building had five windows for natural light. There was no air conditioning, heating, or insulation in the building. The averages for AE activity, temperature, and relative humidity were plotted for each sensor per hour per day for active and inactive logs. The linear association of AE activity and temperature per hour per day was also determined and plotted.

Within an average 24-hour day, AE activity was lowest during the morning, increased in the afternoon, peaked in late afternoon (6 pm), and then declined until mid-morning. For one of the logs (#1), a second peak of AE activity was recorded late into the evening at midnight. Temperature was significantly correlated with the rise and fall in AE activity; warmer temperatures were associated with increasing activity. Relative humidity was not statistically correlated with AE activity. Seasonally, AE activity (i.e. feeding activity) was highest during the warmer spring and summer months. However, an increase in daytime temperature or a sudden heat wave, even in January and February 2009, resulted in an increased burst of AE activity.

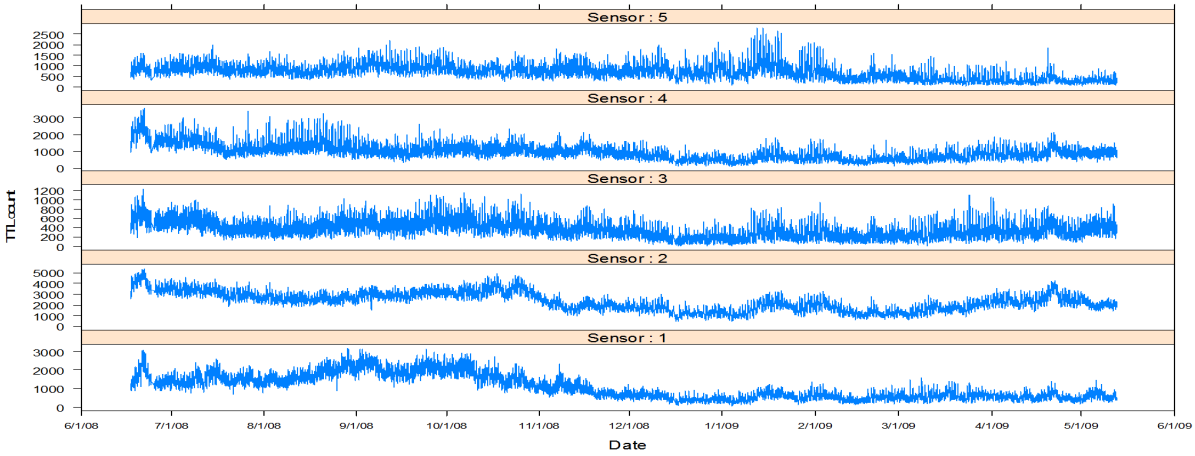
Knowledge of optimal times for drywood termite feeding will be important for evaluating termite inspections. Traditional inspections are based on visual searches for damaged wood or pellets. The data from this study suggest AE readings for active colonies could be enhance by heating the wood to at least 25^{o} C prior to inspection to stimulate feeding, even in winter. Understanding the underlying mechanism that controls the cyclic pattern will require additional studies that include the exclusion of natural light, running additional tests with naturally infested logs at constant temperature and humidity,

and the modification of the AE system collection hardware and software to separate locomotion and communication AE activity from feeding AE activity.

A practical application of this research should result in improved inspections by knowing the times of the day and year when drywood termites are most active for pre- and post-treatment evaluations of remedial treatments.



The mean hourly AE event count for each sensor (1-5) that occurred during the twenty-hour diurnal cycle. The heavy dark line is the mean AE event count. The red line is the temperature trace during the same 24 hour diurnal cycle measured in centigrade (°C).



The seasonal mean hourly AE ring down count (TTL) for each sensor (1-5) that occurred from June 2008 to May 2009.

Conclusions

During the last three years, our laboratory has had access to, and used, some of the most sophisticated termite detection equipment and technology commercially available and beyond. We have produced results that have pushed the boundaries forward on our understanding of drywood termites, their biology, and ecology. Locating drywood termites and their treatment has always been challenging, and with the ushering in of the new decade, the industry has the additional challenges of continuing education on IPM and heightened awareness of water and air quality. How all these challenges are met by

the industry are complex. However, we are optimistic that all challenges will be met. We are prepared and willing to execute bold new investigations that, along with the industry, continue to insure that consumer protection is paramount.

Laboratory and field evaluations of detection devices for drywood termites, *Incisitermes minor*

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Abstract

Laboratory and field investigations were conducted on termite detection technologies that included fiber optics (borescope), acoustic emissions (AE), infrared, and X-ray.

For laboratory investigations, methods were developed for bench top testing of the borescope. Additional methods were designed to calibrate X-ray and infrared camera for viewing artificially and naturally infested boards containing drywood termites. For the borescope, seven categorical items were presented to seven laboratory participants in 5.5 cm clear plastic dishes. The design included the added features of being able to differentiate ants from termites, drywood termite fecal pellets from plant seeds, and pellets from debris. The ability to identify untreated checks (empty plastic dishes) was also included in the design. All participants had a high level of successfully identifying items presented to them in plastic dishes, at least 90%. Similarly, the success rate in correctly identifying untreated checks among participants was 100%. Laboratory investigations produced procedures and techniques to attempt field investigations involving infrared and X-ray. For X-ray, procedures included exposure time (pulse rate) and post-processing and enhancement of images obtained. For infrared, procedures were aimed at creating a temperature gradient between drywood termite gallery architecture and surrounding wood.

For field investigations, the Villa Termiti, a 400 ft^2 (37.2 m^2) wooden structure designed and constructed to compare termite detection and treatment technology, was used. The testing protocol included the use of test boards that were active for drywood termites, inactive (damage only), as well as untreated checks (controls). In total, nine boards were searched with microwave, AE, infrared, and X-ray. The objective for these tests was to determine if any of these devices could singularly identify the presence of damage and active termites in test boards. An additional challenge or variable was to determine capability of the infrared and X-ray devices to make detection determinations through wall coverings that include wood paneling, dry wall, and stucco (X-ray only). At the end of field investigations, all test boards were cut into 10-cm sections and dissected to count live and dead termites. Notes were also taken for the presence and location of gallery architecture.

Under the field conditions of the Villa Termiti, only X-ray demonstrated the ability to accurately detect the presence of damage and termites, albeit these results were best for exposed boards. We were unable to determine whether boards were active with either termites or damage using infrared camera, even after heating the boards for 10 sec at 540 ^oC. AE and microwave produced reliable results (all test boards were determined to be active with termites using both of these techniques and were verified active upon dissection). Plywood, drywall and stucco coverings were not tested for AE and microwaves devices because previous experience in the field has shown that those technologies are impeded by wall coverings due to thickness or difficulty in drilling sensor-testing holes. Results using a borescope in blind tests presented to seven participants were mixed and highly variable depending on the number of pellets, presence

of installation, and the nimbleness among participants to bend down low to manipulate small drilled holes and tight wall void space among participants. For infrared and X-ray, several hundred images of laboratory boards containing termites and actual real-world field conditions have been created and stored in a photo solon.

At this time we cannot recommend a single detection device that can reliably and with a high degree of certainty say that termites are active in walls, especially for high locations (vaulted ceilings) and when wall coverings are in place. No, there is not a single "silver bullet." The best option for drywood termite or any other wood destroying insect inspection is a trained operator with 20 years of field experience and permission to open walls for further inspection. The next best option would be after identifying a wall or better yet a single board, to use an AE device with a subsurface sensor (small wood screw sensor) to determine the board is active for drywood termites. A second device, this time a portable microwave sensor, can be passed held against the board or even drywall (be careful not to hold the device, best to use a tripod to minimize false positives due to vibrations) to aid in identifying the exact locations of galleries. This detection strategy works best for local treatment, where the exact knowledge of active boards and gallery architecture is critical to success.

Background

Economic importance. Surveys of structural-pest inspection reports in California reveal that drywood termites (Family, Kalotermitidae) to have a significant economic importance to homeowners and the pest control industry. There are eight species of drywood termites found in California, among those *Incisitermes minor* (Hagen) has the greatest economic importance (Su and Scheffrahn 1990). Each year, more than 1 million wood-destroying pest inspection reports are conducted in California (Brier et al. 1988). Visual evidence of drywood termites contained in the industry reports suggests drywood termite infestation rates vary from 0 to 46.8% depending on county and location within structure (Ebeling and Wagner 1964, Brier et al. 1988). Costs for control and damage from drywood termites represent 5 to 20% of the > $300 million annually spent in California (Brier et al. 1988, Lewis et al. 2004). Variation in the construction and age of structures pose a formidable challenge to pest control operators (PCOs) when conducting inspections for wood-destroying pests, including drywood termites (Brier et al. 1988).

Visual inspections. Visual searching and probing of wood, including the use of flashlight and metal probe for evidence of drywood termite infestations, is the dominant means of inspection (Scheffrahn et al. 1993). The effectiveness of visual searches for drywood termites for California conditions is unknown. Fecal pellets that are hexagonally shaped in cross-section also are considered diagnostic for the presence of drywood termites and frequently looked for during inspections (Smith 1995). For some species of drywood termites, the number of pellets produced has been used to demonstrate efficacy of chemical treatments (Scheffrahn et al. 1997) and to estimate the size and age of drywood termite colonies (Grace and Yamamoto 2009, Grace 2009).

Alternatives to visual searches. There are at least seven detection devices and methods proposed as alternatives to visual searches. They include acoustic emission devices, electronic odor detectors, dogs, fiber optic devices, infrared, microwaves, and X-ray. They all claim high levels of successful detection of termites; however, few have been scientifically tested (Potter 1997; Lewis 2003; Lewis et al. 2004; 2005). The paragraphs that follow will review the literature for the seven of the drywood termite detection techniques mentioned above.

Acoustic emissions. Termites produce vibrations in wood while feeding and by alarm calls from the head banging of soldiers. Some of these vibrations can be heard by humans, especially when the sounds are amplified by microphone (Emerson and Simpson 1929, Pence et al. 1954, Wilson 1971, Stuart 1988, Kirchner et al. 1994). Several genera in the Kalotermitidae produce audible sounds (Emerson and Simpson 1929). These sounds are produced during feeding (Matsuoka et al. 1996) and by vibratory movements of workers (Leis et al. 1992, Maistrello and Sbrenna 1996). The earliest commercial listening device for termite feeding was an INSECTA-SCOPE. However, no data are available on its performance. Newer technology that amplifies and records termite-feeding vibrations is acoustic emission (AE). Surface and subsurface probes are available, and successful detection of drywood termites in laboratory settings is at least 80% (Lewis and Lemaster 1991; Lewis et al. 1991; Scheffrahn et al. 1993, 1997; Lewis and Haverty 1996; Lemaster et al. 1997; Lewis et al. 2004, 2005). Wall covering can impede sensor and AE performance. Distance in detection is limited to ≈80 cm along the length of a board and < 8 cm across the grain (Scheffrahn et al. 1993). Excessive background noise can also result in falsely reporting termite activity. AE detection equipment is commercially available, although availability is very limited.

Electronic odor detectors. All termites produce gases during their metabolism of nutrients. The most abundant gas produced is methane (Khalid et al. 1990). Commercial devices have been developed to assist with the termite detection process using methane gas detectors (Lewis et al. 1997). However, when tested under laboratory conditions, at least one commercial methane gas detector was unreliable in detecting subterranean termites. There are no studies that mention the success rate in detecting drywood termites. Future innovations are needed in electronic odor detectors to reliably detect the presence of termites in structures under field conditions.

Dogs for termite searches. Dogs have also been used to assist with drywood termite inspections, although scientific studies verifying the effectiveness are few. Several breeds of dogs have been trained, beagles being the mostly frequently used (Lewis et al. 1997). The mode-of-action used by dogs in finding termites, sound or odor or both, still needs further research (Lewis et al. 1997). For California, the effectiveness of beagles was mixed but only included subterranean termites. Drywood termites were not included in the laboratory investigations (Lewis et al. 1997). However, drywood termites were included in laboratory trials conducted in Florida and the success rate for two breeds of dogs (beagle and German shepherd) in identifying plastic containers containing drywood termites, *Cryptotermes cavifrons* Banks and *Incisitermes snyderi* (Light), was 88.8% (Brooks et al. 2003). False positives, canines response to containers without drywood

termites was < 1%. Currently, there are few commercial firms that train and provide dogs to assist with termite inspections.

Fiber optic devices. Optical borescopes use visable light passing through a hollow tube as a means to view termites and damage hidden behind walls. A small hole must be drilled into walls to allow viewing. Fire blocking, insulation, and viewing through a fish-eye lens may impede the inspector's view. Optical borescopes are currently marketed; however, their efficiency in the detection of drywood termites has yet to be scientifically tested.

Infrared. Infrared rays are part of the electromagnetic spectrum, nearest red in the visible range. Their existence has been known for several hundred years (Maldague and Moore 2001). Although invisible to the human eye, infrared energy has a penetrating heating effect and is easily felt when encountered. Most objects, living or not, give off infrared heat, whether internally generated or reflected. Initial uses of infrared were for military surveillance, as early as World War II (Maldague and Moore 2001). Today, there are many nonmilitary uses and include measurement devices, binoculars, night viewing for hunting, etc. In structures, infrared devices have been used to find faulty electrical connections and heat and water leaks in walls and roofs (Maldague and Moore 2001, Tobiasson 1994). A more recent use includes termite detection (Lewis 2003). Commercial termite detection models are available. Some testimonials and demonstrations on the termite detection ability of infrared exist (Anon. 2000); however, the effectiveness in finding termite infestations has not been scientifically tested. A possible drawback in the commercialization of this technology as a drywood termite detection tool is that the small temperature difference between the termites and wood, at least at ambient level, may hinder this technology in locating infestations (Carroll 2002).

Microwaves. Microwaves comprise the frequency range 0.3 to 300Ghz and lie between radio and infrared region of the electromagnetic spectrum (McMaster et al. 1986). Microwaves are commonplace in our daily lives and their commercial applications are many and varied and include communications (McMaster et al. 1986), cooking (Steele 1987), internal scanning of wood (Martin et al. 1987), radar (McMaster et al. 1986), termite control (Lewis and Haverty 1996, Lewis et al. 2000), termite detection (Peters and Creffield 2002, Evans 2002), wood drying (Chen et al. 1990), and detection and pest management of stored product pests (Watters 1976; Nelson 1973, 1977; Regan et al. 1980; D. Hall 1981; D'Ambrosio et al. 1982; Nelson and Payne 1982; Tilton and Vardell 1982a,b; Philbrick 1984; Del Estal et al. 1986; Rosenberg and Bögl 1987; A. Hall 1988; Locatelli and Traversa 1989; Shayesteh and Barthakur 1996, Mankin 2004). Published papers using microwaves have been reported for several species of termites (Peters and Creffield 2002, Evans 2002). Success in detection of drywood termites (*Cryptotermes brevis* (Walker)) using microwaves (TERM_A_TRAC™) was 86% based on laboratory studies (Peters and Creffield 2002). The maximum detection depth reported was 35 mm for untreated pine test boards and 25 mm treated pine and hardwood. However, water in wood, wall coverings, and excessive wind and motion can lead to false positives results for live termites.

X-ray. These penetrating rays are part of the electromagnetic spectrum that is nearest ultraviolet rays. They are invisible, have smaller wavelength, and have a higher frequency (Hz) compared to the visible part of the spectrum (Ness et al. 1996, Maldague and Moore 2001). There are many commercial applications for X-rays and include dental, medical, military, and security applications, as well as nondestructive evaluation of materials (Martz et al. 2002). X-ray energies have the ability to penetrate nearly all materials (Martz et al. 2002). These penetrating rays have also been used to nondestructively view insects in hidden locations (Fisher and Tasker 1939; Berryman and Stark 1962a,b; Berryman 1964; Davies et al. 1988; Kim and Schatzki 2001), and internal structures of insects (Westneat et al. 2003). For viewing insects hidden in wood, X-rays have been used for at least seven decades (Fischer and Tasker 1939, Kim and Schatzki 2001). X-rays have also been used to view several structural infesting beetle pests (Fisher and Tasker 1939, Suomi and Akre 1992). Only recently has the potential use of X-ray in detecting drywood termite infestations been explored (Lewis et al. 2005). Older X-ray technology was cumbersome to use, while newer technology is more portable. Processing of images is accomplished with lasers that produce digital images that are enhanced from computer software.

The purpose of our research was to explore, under laboratory and field conditions, the detection performance of AE, fiber optic borescope, infrared, microwave, and X-ray technology for identifying and delimiting drywood termite infestations. Canine and electronic odor detection was not included in this study due to the inability to secure a dog and device for laboratory and field investigations.

Materials and Methods

Objective 1. Determine the effectiveness of AE, fiber optics scope, infrared, microwave and X-ray, in finding drywood termite infestations in naturally infested boards.

Bench top laboratory investigations involving the borescope. A fiber optics scope (ProVision, CML Innovative Tech., Inc., Hackensack, NJ) was used in a laboratory bench top setting to determine its robustness in detecting visual evidence of drywood termites. The device was provided by a large pest control company in the State and has seen field use in real world inspections for wood-destroying organisms. For laboratory evaluation of the borescope, commonly encountered arthropod evidence found in walls were used that included drywood termite pellets, dead drywood termite workers, dead carpenter ants and dead Argentine ants (Fig. 1). For untreated checks on the device's performance, materials included tested its capability in distinguishing detail (drywood termite pellets with hexagonal sides versus straight grains of brown rice) and color (drywood termite pellets versus raw sugar) (Fig. 1). Several grams of material were poured into 5.5-cm diameter plastic dishes to be viewed from a bench top worktable. A 42-question identification quiz was developed using all test materials, multiple times, and was administered to seven participants from the lab. For testing, the borescope was affixed to a board (Fig. 2). A participant's view of material was blocked during quiz administration

by a piece of cardboard. The borescope board could freely move to allow better viewing of material.

To visually document borescope capabilities, standard digital images were taken of all materials and were also taken through the borescope eyepiece from two viewing distances, 2 cm and 4 cm (Fig. 2). Digital images were also taken of pellets, through the borescope eyepiece, from a 1-meter viewing distance.

Analyses methods. Quiz answers and scores were recorded and into an EXCEL file. Standard descriptive statistics using Chi-squared tests were used to describe to determine differences in the correct identification percentage of materials among participants. Digital images were stored in a photo salon.

Villa Termiti investigations. For laboratory portion of the study, all testing of detection devices was conducted in the Villa Termiti. This simulated-field testing structure was built in 1993 from funding provided by the Structural Pest Control Board (RFP 91-001) to evaluate nonchemical methods of control for drywood termites. The building is 6.1 by 6.1 m (37.2 m^2; 154 m^3), has symmetrical sides, and composed of attic, drywall area, and subarea (Lewis and Haverty 1996). Testing within walls consisted of exterior (containing installation material) and interior (no installation material) components. All wall cavity spaces had fire blocking wood members. Robust testing conditions were built into the structure and include, varying wood species, lumber dimensional sizes, and wall coverings.

Boards. Dozens of naturally infested boards containing drywood termites were collected from at least 10 cities around California and are stored and maintained at the University of California Richmond Field Station in, Richmond, CA. Using AE technology boards were determined to contain active drywood termites by drilling 0.3 cm diameter holes and inserting the Termite Tracker sensor probe (Dunegan Engineering, Midland, TX) roughly 1.9 cm deep and taking three one-minute readings for feeding episodes every 57.2 cm of board length. Five boards that had an average count of 20 per minute or higher were eligible for the study; however, only three were randomly selected for further processing. For each active board selected, board dimensional measurements (length, width, and height), wood species, and presence of paint or stain were also recorded. Lastly, a digital image was taken of each longitudinal side.

An additional six boards were randomly chosen as controls: three undamaged boards and three damaged, but with no live termites. Damaged, but inactive (DI) boards were used to assess the ability of detection equipment to determine presence of damage alone. Undamaged boards (controls) were used as a standard of comparison. For DI boards, after taking their dimensional size (length, width, and height), wood species, presence of paint or stain, and digital images for all sides, they were placed into a 110 C^o oven over night (24 hours) to kill any drywood termites. One active, one DI, and one control board were grouped together as one group and replicated three times (Table 1). These boards were used for tests involving AE, infrared, microwaves, and X-ray.

Boards for each group were randomly positioned into separate stud bays in the Villa Termiti. Two small brackets and/or wood screws were used to affix each board to the plywood backing in the center of the stud bay, roughly 0.4 to 0.9 m up from the floor level depending on the length of the board. The side of the board that revealed the most

visual evidence of termite feeding damage faced outward towards the main living space, the area that contains all the doors and windows. A digital image was taken for each board in its final stud bay position (Fig. 3).

Methods involving AE. A 0.3-cm diameter hole was drilled roughly 1.9 cm deep into the test board for every 57.2 cm of board length. Using three different AE devices, a probe was inserted into drilled holes for active, DI, and control boards. Three one-minute readings for feeding episodes were taken simultaneously. To account for device variability every AE device was used on each board (Fig. 3). For every AE hole in an active board, a total of nine AE readings were taken, *i.e.* three one-minute AE readings from each of the three AE devices.

Analyses methods. All AE counts were entered into an EXCEL spread sheet to be analyzed using ANOVA and multiple comparisons tests (SAS Institute 1994) were conducted to compare the mean AE counts between active, DI, and control boards.

Methods involving fiber optic borescope. For field-testing, twenty-eight voids were randomly assigned as either representing an exterior wall (with insulation) or interior wall (without insulation) wall. Interior and exterior wall voids were assigned one of seven treatments. Testing variables for the study included; debris (dirt, arthropod parts, cobwebs, and wood shavings), debris with pellets, 0.5 g of pellets dropped from either 1.5 cm or 1 m, 2 g of pellets dropped from either 1.5 cm or 1 m and nothing. Each treatment was assigned two times within exterior (containing installation material) and interior wall groups (no installation material). Stud bays were closed, to sufficiently block view, with a combination of cardboard and ply board or drywall (Fig. 4). For borescope insertion, holes were drilled at the center of each void 5.1 cm above the mudsill plate. Seven participants from the laboratory staff conducted the borescope field test. Participants were provided with a borescope, a metal probe, and a piece of paper to identify and record the presence or absence of pellets and other items in the voids.

Analyses methods. Quiz answers and scores were recorded and into an EXCEL file. Standard descriptive statistics using a Pearson's Chi-squared test were used to assess differences in identification of materials among participants.

Methods involving infrared. All thermal imaging of test boards were conducted using a ThermaCam™ BX320 **(**Flir Systems, Wilsonville, OR). A thermal image was taken, at ambient temperature, before each heating set. Using a hand held heat gun (Heat Gun™ HT 1000, Wagner, Minneapolis, MN), test boards were heated, then viewed with the infrared camera for the presence of damage. The heat gun had two settings for heating; 400 ^{o}C and 540 ^{o}C. The maximum setting (540 ^{o}C) was used and involved heating two-foot sections (0.6 m) of the nine test boards with two different heating durations: 1) one-pass of the heat gun for several seconds, and 2) ten seconds. After heating, thermal images were taken with the infrared camera. The same heating and picture process was repeated for test boards covered with 1.3 cm plywood and 1.3 cm drywall.

Analyses and presentation of results. Images were collected and stored in a photo salon for visual (qualitative) analysis of anomalous heating and/or cooling patterns.

Methods involving microwave. For this test, a TERM_A_TRAC™ motion detector (TERM_A_TRAC™, Sydney, Australia) was used (Fig. 5). Test boards were measured lengthwise into 10 cm sections. If a board was more than 12.7 cm wide, it was also divided in half vertically. Using clamps, the microwave horn was placed flush and immobile against each 10-cm section of board (Fig. 5). The LCD display for the TERM_A_TRAC™ was fitted (using adhesive tape) with a 50-mm metric ruler that allowed us to visually record how far (in mm) the lighted indicator bar moved across the viewing screen. For each 10-cm section of board, the microwave ran continuously for 75 seconds, allowing for one 15-second equilibration period and four 15-second replicates. All termite motion data was video recorded using an HDV-Handycam® (Sony Electronics Inc, San Diego, CA).

Analyses and presentation of results. All videotaped results were reviewed using a DVD player (Sony Computer Entertainment, Inc., Foster City, CA). The maximum distance the indicator bar moved was recorded for each second within the 15-second replicate. These numbers were entered into an EXCEL spread sheet. An ANOVA and a multiple comparisons test were used to compare the maximum mean distance moved by the indicator bar for active, DI and control boards.

Methods involving X-ray. A portable x-ray device (XR-200 X-ray Source, Golden Engineering, Inc., Centerville, IN) was tested for viewing termites and damage for exposed boards and through wall coverings (Figs. 6 and 7). Laboratory testing and training with this device allowed the laboratory staff to estimate the necessary number of pulses (exposure time) for quality images to be viewed and analyses for the presence of drywood termites, gallery system, and damage.

For each test board, full-length X-ray images were taken of exposed boards (no coverings) and of boards through wall coverings, which included 1.3-cm ply board, 1.3-cm drywall, and stucco exterior with chicken wire. X-ray images were obtained of the ply board, drywall and stucco exterior alone. For complete simulation of field conditions, X-ray images were also taken of the wood floor (through the 1.3-cm thick wood floor only and through a floor joist) and roof (through the roof only and through a roof rafter). All images were processed using image enhancement software (Logos Imaging, Richmond, IN) to increase contrast for viewing damage and termites. Images were stored in a photo salon for further review and processing.

Analyses and presentation of results. This was a descriptive study, images were produced and compared to those through varying wall covering materials, digital camera images at ambient light levels, and images obtained from an infrared camera.

Methods involving board dissection and termite counts. At the conclusion of all device and detection testing in the Villa Termiti, all nine test boards were cut into 10-cm sections, pictures were taken of the top and bottom cross sections, and then pieces were dissected and searched for drywood termites (dead and alive).

Analyses methods. All count data were stored in an EXCEL spread sheet as section number per board. For devices that have quantifiable variables, analysis was presented as means per 10 cm of board linear distance. Pictures of cross sections were stored in a photo salon.

Results and Discussion

Bench top laboratory investigations. The results from the bench top investigations involving the borescope and discriminating among six types of materials contained in plastic dishes suggest a high degree of successful detection among all the seven participants and their performances were found to be similar ($X^2 = 8.17$, df = 6, P>0.10). Participants correctly identified termites, pellets, ants and debris at least 90% of the time (Fig. 8). Materials tested were correctly identified at a higher level than by chance alone (P < 0.05, Table 2). Similarly the level of success in discriminating among the untreated checks (empty dishes) was also very high, 100% (Fig. 8 and Table 2). The only treatment that did not achieve a high level of successful detection was for the confused for pellets category, a mixture of either celery or fennel seeds, 69%. Realistically, celery and fennel seeds do not appear in walls; however these results lead to the interpretation that even in the best-case-scenario conditions of laboratory bench top experiment in plastic dishes, discriminating the appearance of drywood termite pellets in debris based on seeing and counting the hexagonal sides can be challenging.

Field tests with borescope in the Villa Termiti. The successful detection of drywood termites and pellets under field conditions was considerably lower compared to the bench top results (Figs. 8 and 9; Tables 3 and 4). The highest successful detection rates were for debris in wall voids (86%) and for untreated checks (75%, empty wall voids) and both were significantly higher compared to 50% level of chance ($X^2 > 3.841$, df = 1, P < 0.05, Table 3). Participants identified two grams of pellets, dropped from either 1.5 cm or 1 m, at a higher rate than 0.5 g of pellets, from both heights. Data suggest that the borescope is not sensitive for newer and/or low-level infestations. There were considerable differences in overall detection success among participants (Fig. 9). Interestingly, the lower successful detection rates, participants C & G, at 36% and 50%, were the two senior scientists and entomologists in the lab. The highest level of correctly identified items (79%) was for one of the youngest lab members, a chemist and physicist by training, not an entomologist. At least in the Villa Termiti field tests, being nimble and having the ability to bend down low to manipulate the borescope into small drilled holes and within tight wall void spaces proved more useful that years of studying and writing termite papers. The important point from this test is that detection success is always lower and more demanding under field conditions when compared to laboratory manipulations. We suspect that under real-world field conditions, inspections with the borescope require flexible equipment, optical viewing lens magnification capabilities, inspection and equipment training, some manual dexterity, and lastly, homeowner and client permission to drill a sizable hole in their walls.

X-ray investigations. The greatest detail obtained among detection devices investigated during the study for the presence of live drywood termites and their exact location within boards was achieved by use of X-ray. The photo salon of X-ray images (600 individual images) taken during the course of the investigations, revealed startling details, even to the imaging of individual termites (although rare). Best-case scenario examples of X-ray images contained in the salon can be seen in Figure 10. The scanned

X-rays are large 8-MB files and some detail is lost when printed. On average the placement of X-ray emitting source was ≈1 m away from the targeted area of board under investigation. The amount of time needed for the emitter to direct X-rays at targeted areas within boards varied from 10 sec to 30 sec depending on the dimension size of the area and presence of wall covering. Images produced by X-ray were best when test boards did not have wall covering (Fig. 3, Fig. 10). For some severely damaged boards, you could still see some gallery architecture through plywood and drywall, but limited damage could be seen through stucco exterior (Fig. 10). However, to obtain this imagery required considerable effort. At least 2 hours per spot (including image editing), ability to touch the spot (no vaulted ceilings), and the area viewed per spot was limited to at best two 8'' by 17'' sheets of film. Our research team also had to devise adhesive system to attach X-ray imaging films to wall surfaces as well as to attach and aim the portal X-ray device in hard to reach and/or cramped areas.

This is the first investigation on the potential use of X-ray to view drywood termites and their galleries. Earlier published investigation focused on wood boring beetles (Fischer and Tasker 1939; Berryman and Stark 1962a,b; Berryman 1964; Suomi and Akre 1992) and stored product pests (Kim and Schatzki 2001). Realistically, because of equipment costs ($25,000), size and weight of scanner (100 pounds, 45 kg), high level of training to operate equipment, and large safety buffer zone (10 feet (3 m) behind, 36 feet (11 m) to the sides and 100 feet (30 m) in front of the emitter) needed to safely operate the equipment under field conditions, it is doubtful that this penetrating form of termite detection will be realized for large-scale adaption by the industry in California. An additional limitation is that with current technology there is considerable subjectivity in determining what "is" and "is not" termites and damage. An additional technological feature needed is computer-assistance software based on pixel points for images that will alert the operator to the presence and locations of termites.

Infrared investigations. Laboratory and field investigations using infrared proved very limited in the successful detection of drywood termites in test boards. Infrared images at ambient temperature level did not reveal any discernable pattern of termite damage or underlying gallery structure (Fig. 11). After applying heat (5-10 seconds at 540 ^{o}C) the resulting image appears to reveal damage (Fig. 11). However, when compared to digital images, the pattern seen corresponded to knots, peeling paint, and chips in the wood surface and not termite damage. Blemishes to the wood surface caused by paints, knots, and chips all can produce changes in infrared signals perceived by the camera. When wall coverings were added (wood paneling, drywall, or stucco) no visual evidence for drywood termite damage or galleries could be seen in images produced (Fig. 11).

From our field investigation (Villa Termiti tests), the use of infrared was limited to exposed boards that required heating. The underlying mechanism in heating boards and viewing damage is that a differential or gradient of temperatures is produced in test boards, for example a gallery is filled with air, which acts as insulation causing the wood above to heat up more compared to intact bulk wood surrounding a gallery. It is this difference in temperature or artificially produced temperature gradient that is viewed by the infrared camera and produces the pattern of damage seen by the operator.

There are few studies in the scientific literature that confirm the use of infrared for termite detection. Gilberg et al. (2003), presented only empirical observations and some infrared images of subterranean termite damage for a church in New Orleans. Since subterranean termites, especially the Formosan subterranean termite (*Coptotermes formosanus* Shiraki) uses wet mud and water to build their galley system and carton nests, a temperature gradient is produced between wood containing high levels of water to ambient levels for very dry structural wood members. Because drywood termites do not require water for nest building nor galley formation, a temperature gradient is not created to exploit infrared imaging.

Perhaps future features in infrared detection could include means of creating a temperature gradient that better differentiates drywood termites and their galley system in naturally infested structural lumber. Although such a technology has been described as a patent (Lee 2008), such a system is not currently commercially available. An additional technological advancement needed that could enhance the potential use of infrared for drywood termite detection is the creation of software that post-processes the pixel image and automatically alerts or notifies the operator when and where drywood termites and their galleries are located within infrared images and infested areas.

Microwave investigations. Microwaves were highly successful in identifying termite motion, albeit only for a small area within test boards. The motion of termites within boards containing live termites was significantly greater than untreated checks (2.3 mm/15 sec versus 0.26 mm/15 sec versus 0.05 mm/15 sec; $F= 104.4$; $df = 2,8$; $P <0.0001$; Table 5). Boards active with termites were at least an order of magnitude greater in motion activity compared to untreated checks. However, the area where termite detection was reliable was 5 by 6.5 cm (32.5 cm^2; 5.0 in^2) and 3.5 cm (1.4 in) deep; rather small considering the thousands of ft^2 (hundreds of m^2) of wood that drywood termite can potentially infest in homes. For untreated checks (control and damaged inactive boards), although statistically not significant when compared to boards active with termites, these still had some measureable motion and suggest sources of background motion that include wind and vibrations from foot-traffic could lead to false positives.

Our findings support previous laboratory studies that microwaves have potential applications for non-destructively assessing the presence of active infestations and active galleries within boards and buildings (Evan 2002, Peters and Creffield 2002). Additional findings from our investigations and experience with these devices point to the need for devices that have more than a single sensor, greater area of detection, and flexibility in equipment design that can accommodate tight angles and corners in structures under field conditions. As a research tool for studies that involve termites, we would like additional features that include a digital number that reflects active termites detected versus a moving bar over a digital screen, as well as storage and software capabilities to retrieve field collected data for further processing and statistical analyses.

Acoustic emission (AE) investigations. Field tests conducted in the Villa Termiti continue to support AE technology as a useful tool in determining whether boards are active or not for drywood termites. When compared to untreated checks (control and DI boards), the average AE counts per minute for active boards was 117.6 compared to 0.9 and 0.6). These differences were statistically significant ($F = 6.78$; $df = 2, 8$; $P < 0.03$).

When the boards for the current study were dissected, the success rate in predicting live termites among test boards was 100% (Table 6). There are at least eight papers in the published literature report similar detection success when using AE technology (Lewis and Lemaster 1991; Lewis et al. 1991; Scheffrahn et al. 1993, 1997; Lewis and Haverty 1996; Lemaster et al. 1997; Lewis et al. 2004, 2005). However, the detection results for the local treatment field study also conducted by our laboratory and funded by the Structural Pest Control Board, was not able to find statistical significant differences when comparing pre- and post-treatment AE counts (Lewis et al. 2009). The exact reasons for the discrepancies in AE finding among the detection and local treatment field study are not fully know at this time, but probably involve the scale of testing and robustness of detection equipment for actual field conditions. Additional investigations under field conditions encountered by PCOs in the California include diversity in construction, vaulted ceilings, wall coverings, and taking detection reading during the colder winter months. Also helpful would be to remove boards from in-place field conditions from homes with termite activity and subsequently treated, then returning them to the laboratory for detailed dissection to better correlate AE readings and the actual presence of termites.

Future needs in termite detection technology. There is sometimes a mind set that a "silver-bullet" can solve the world's problems or needs. However, when detailed investigations are launched, sometimes at great expense, these "silver-bullets" rarely pan out. Such is the case with termite detection. During the last three years our laboratory has had access to and used some of the most sophisticated termite detection equipment and technology commercially available and beyond. No, at this time we cannot recommend a single detection device that can reliable, and with a high degree of precision, say that termites are active in walls, especially for high locations (vaulted ceilings) and when wall coverings are in place. The best option for drywood termite or any other wood destroying insect inspection is a trained operator with 20 years of field experience and permission to open walls for further inspection. However, you can't always open walls and some infestations are just hard to find. The next best option would be after identifying a wall or better yet a single board, then use an AE device with a subsurface sensor (small wood screw sensor) to determine the board is active for drywood termites. A second device, this time a portable microwave sensor, can be held against the board or even drywall (be careful not to hold the device, best to use a tripod to minimize false positives due to vibrations) to aid in identifying the exact locations of galleries. This detection strategy works best for local treatment, where the exact knowledge of active boards and gallery architecture is critical to success. For whole structure treatment, the exact location and active status of infestation is not as critical compared to local treatments, but any objective measures of treatment success or failure would go a long way insuring consumers that their best interests and protection is the guarantee being provided to them.

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Table 1: Active, DI (damaged but without termites), and control boards grouped together in stud bays that were randomly assigned. The cross-sectional dimensions and length and included for each test board.

			Board Dimensions		
Location	**Board ID**	**Wood Type**	**Depth (in/cm)**	**Width (in/cm)**	**Length (in/cm)**
Bay 1	Active A	Douglas Fir	1.5/3.8	2.4/6.1	23.3/59.2
	DI A	Douglas Fir	1.5/3.8	2.5/6.4	24.5/62.2
	Control A	Douglas Fir	1.5/3.8	3.5/8.9	23/58.4
Bay 2	Active B[1]	Douglas Fir	3.5/8.9	6.5/16.5	54.5/138.4
	DI B	Douglas Fir	3.5/8.9	3.5/8.9	20.5/52.1
	Control B	Douglas Fir	3.5/8.9	1.5/3.8	23/58.4
Bay 3	Active C	Douglas Fir	1.7/4.4	5.5/13.9	15/38.1
	DI C	Douglas Fir	1.5/3.8	5.2/13.2	25/63.5
	Control C	Douglas Fir	1.5/3.8	5.5/13.9	23/58.4

[1]Active A was comprised of 3 boards nailed together. They were not separated in order to protect the integrity of the colony in the wood. The dimensional sizes and length reported represents the average of the three boards.

Table 2. Number right, wrong and percentage of items correctly identified in the borescope laboratory investigation. Participant (total 7) data was pooled for each material. Items identified during the laboratory investigation included drywood termites, confused for termites (brown rice and raw sugar), drywood termite pellets, confused for pellets (celery and fennel seeds), ants, debris and empty containers (controls).

Material	Right	Wrong	% correctly identified	χ_c^2	Significant at $\chi^2_{0.05,1} = 3.8$
Termites	40	2	95%	32.6	Yes
Confused for Termites	42	0	100%	40.0	Yes
Pellets	40	2	95%	32.6	Yes
Confused for Pellets	29	13	69%	5.36	Yes
Ants	38	4	90%	25.9	Yes
Debris	39	3	93%	29.2	Yes
Empty (controls)	42	0	100%	40.0	Yes

Table 3. Number right, wrong and percentage correctly identified for seven treatments presented to seven participants from borescope field tests[1]. Participant (total 7) data was pooled for each treatment.

Treatment[2]	Right	Wrong	% correctly identified	χ_c^2	Significant at $X^2_{0.05,1} = 3.841$
Interior[3]	67	31	68%	7.29	Yes
Exterior[4]	56	42	57%	N/A	N/A
0.5 g of Pellets from 1.5cm	13	15	46%	0.04	No
0.5 g of Pellets from 1m	13	15	46%	0.04	No
2 g of Pellets from1.5 cm	20	8	71%	4.32	Yes
2 g of Pellets from 1 m	20	8	71%	4.32	Yes
Debris	24	4	86%	12.9	Yes
Debris and Pellets	12	16	43%	0.32	No
Untreated (empty)	21	7	75%	6.04	Yes

[1]Field tests conducted in wall stud bays at the Villa Termiti, Richmond, CA.

[2]There was a total of 28 voids checked by each of 7 participants, for a total of 196 voids checked. Half (98) of the voids were interior walls and half were exterior walls. The other treatments are presented irrespective of interior and exterior wall categories, and thus all add to 196.

[3]Interior walls did not contain insulation.

[4]Exterior walls contained insulation.

Table 5. Mean distance (in mm) moved by the indicator bar in the viewing screen of a microwave device[1] during a 15 sec observation period for test boards active with termites, DI (damaged but without termites), and controls (sound undamaged boards without termites).

Treatment	Replicates	Mean distance (mm)	Standard Error
Active	3	2.27	0.14
Control	3	0.26	0.14
DI	3	0.05	0.049

[1]See microwave section in materials and method section and Figure 3 for treatment details.

Table 6. AE and dissection results for three board groups, Active, DI (damaged but without termites) and control boards. Dissection was done nine months after AE readings were taken.

		AE Count		Termite Count	
Treatment	**Replicates**	**Mean**	**Standard Error**	**Average Alive**	**Average Dead**
Active	3	117.605	44.926	291	11
Control	3	0.947	0.42	0	0
DI	3	0.626	0.076	0	6

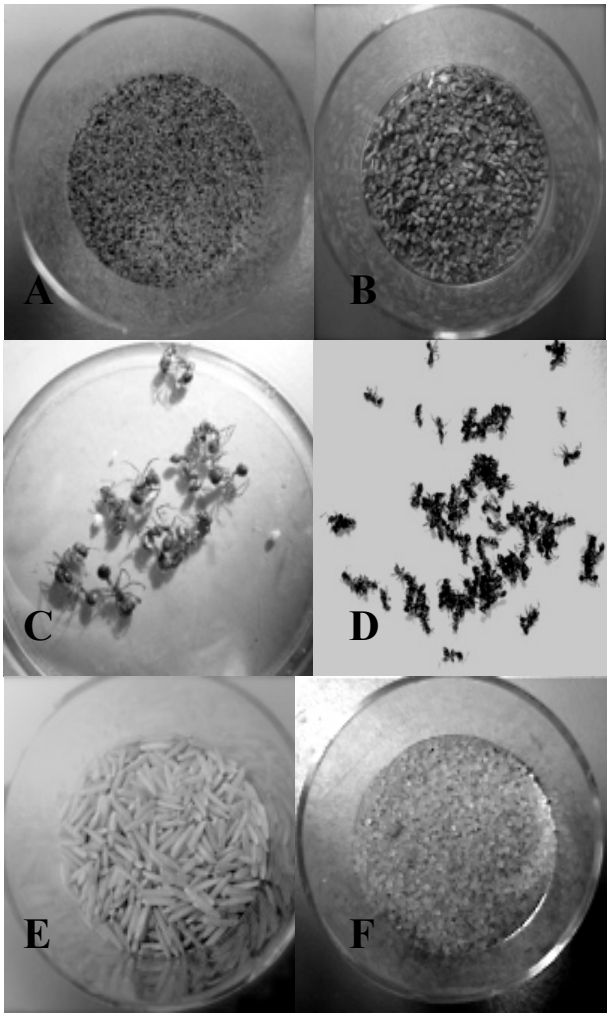

Figure 1. Materials used for bench-top laboratory borescope study: a) drywood termite frass, b) dead drywood termite workers, c) dead carpenter ants, d) dead Argentine ants, e) brown rice, and f) raw sugar. Borescope/fiber optics device used ProVision, CML Innovative Tech., Inc., Hackensack, NJ.

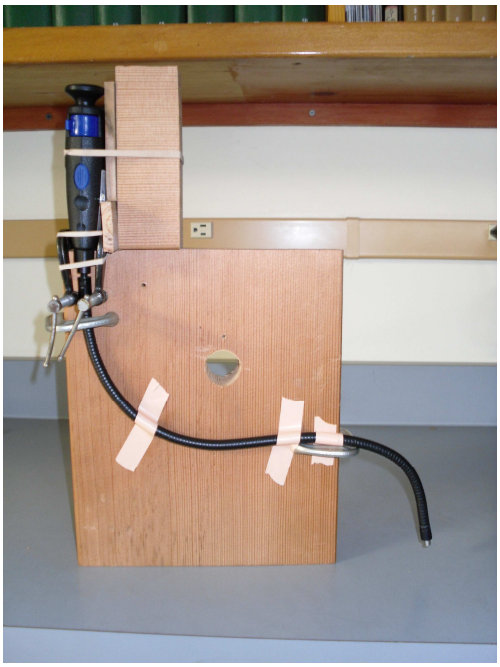

Figure 2. Borescope device and setup used for bench top laboratory investigation on detection accuracy for selected items. For digital images, the end of the fiber optic scope was adjusted to either 2 cm or 4 cm above the counter top. See the methods section for a complete list of materials included in the study for participant viewing.

Figure 3. Stud bay 1, wall voids, and AE equipment used to measuring drywood termite feeding activity in field-collected boards. The board in the left hand stud bay is an untreated control board (no termites or damage), middle board has damage only, no termites, and the far right board active with live drywood termites. The black boxes and sensors with flexible cables are for portal AE detection equipment (Tracker, Dunegan Engineering, Midland, TX).

Figure 4. Materials used in borescope the additional laboratory study: a) drywood termite frass, b) dead drywood termite workers, c) dead carpenter ants, d) dead Argentine ants, e) brown rice, and f) raw sugar. Borescope/fiber optics device used ProVision, CML Innovative Tech., Inc., Hackensack, NJ.

Figure 5. Test boards and portable microwave detection device used for field study. Test site, Villa Termiti Richmond, CA. The two yellow devices in the middle of the image are of a TERM_A_TRAC™ (Sidney, Australia). The left hand side device is the main operating component and the right side component is the sensor. Clamps were used to secure sensor to test boards.

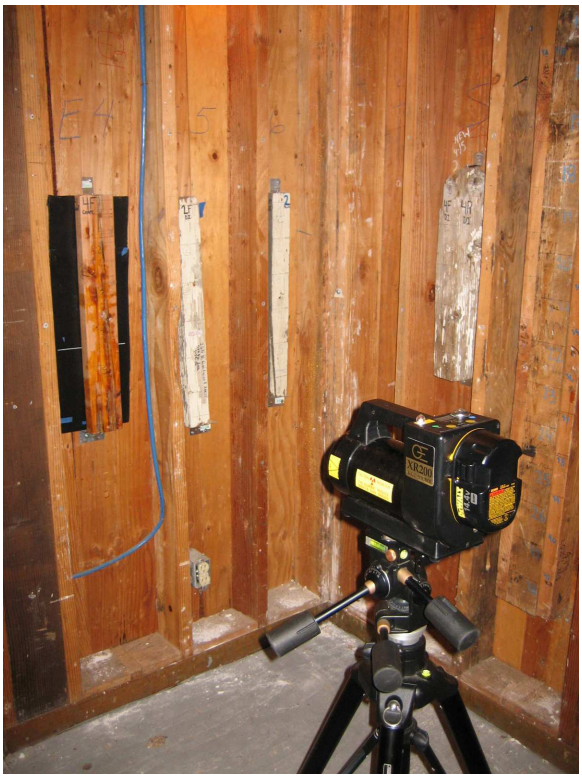

Figure 6. Test boards, imaging plates (in black cover behind far left board) and portal X-ray detection device used for field study. Test site, Villa Termiti Richmond, CA. The black device in the center of the image is a XR200 X-ray emitter (Golden Engineering, Inc., Centerville, IN).

Figure 7. Wall coverings used to test the penetrating ability of X-ray and infrared to view damage and termites contained in field-collected boards. Test site, Villa Termiti Richmond, CA.

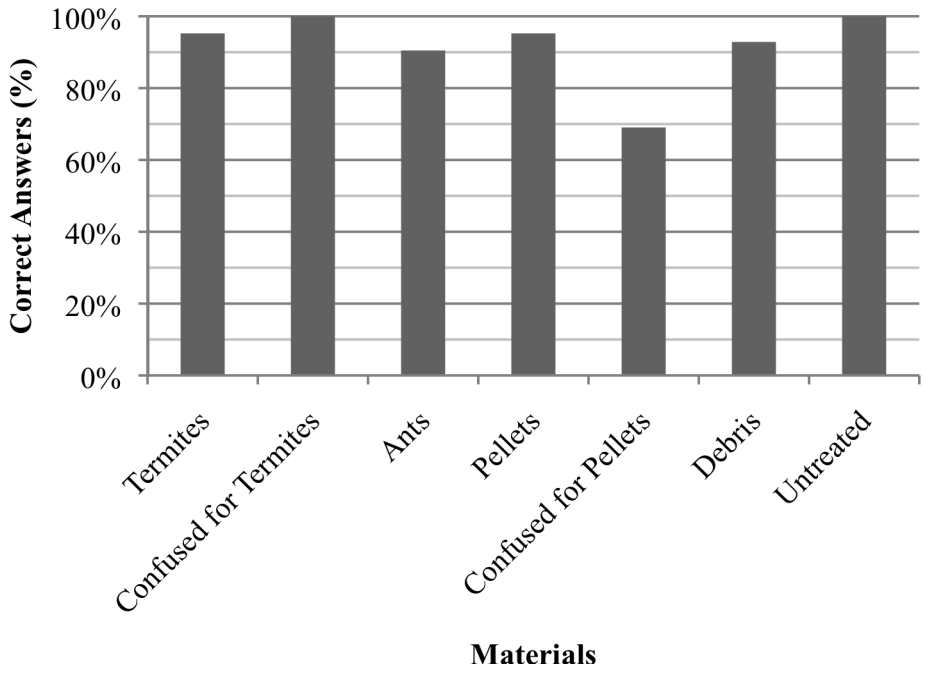

Figure 8. Percentage of correctly identified items using a borescope during laboratory investigations. The items included in the study were drywood termites, confused for termites (brown rice and raw sugar), ants (carpenter and Argentine), drywood termite pellets, confused for pellets (celery and fennel seeds), debris and empty containers (controls). Seven participants were included in the study, all members of the laboratory team.

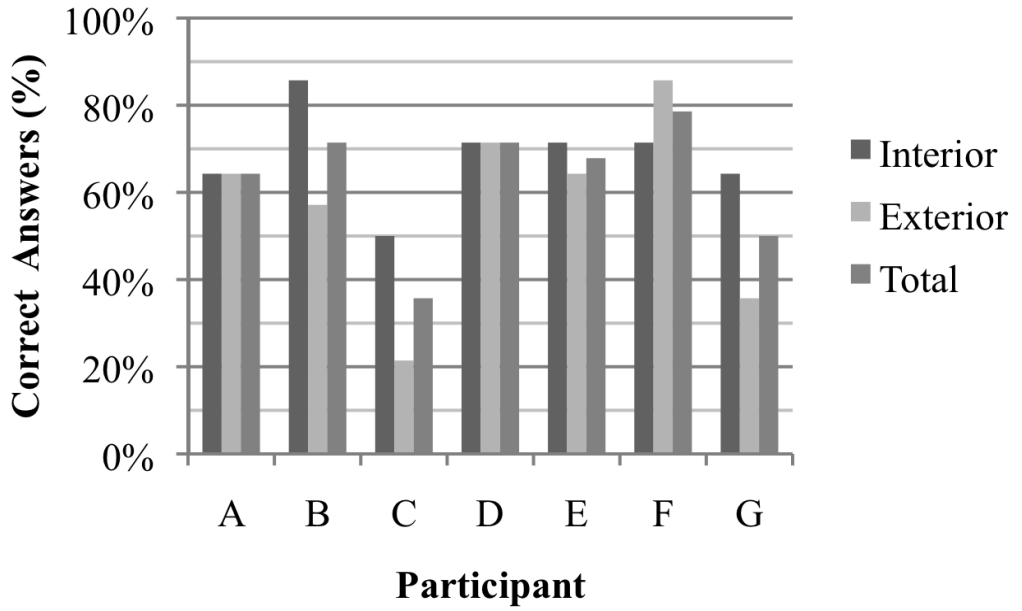

Figure 9. Percentage of correctly identified items, by each participant, using a borescope during field investiations. The items included in the study were debris (dirt, arthropod parts, cobwebs, and wood shavings), debris with pellets, 0.5 g of pellets dropped from either 1.5 cm or 1 m, 2 g of pellets dropped from either 1.5 cm or 1 m and nothing. Seven participants (A through G) were included in the study, all members of the laboratory team. Overall participant performances were found to be different using a proportions comparison test ($\chi^2 = 15.8$, df = 6, $P < 0.05$).

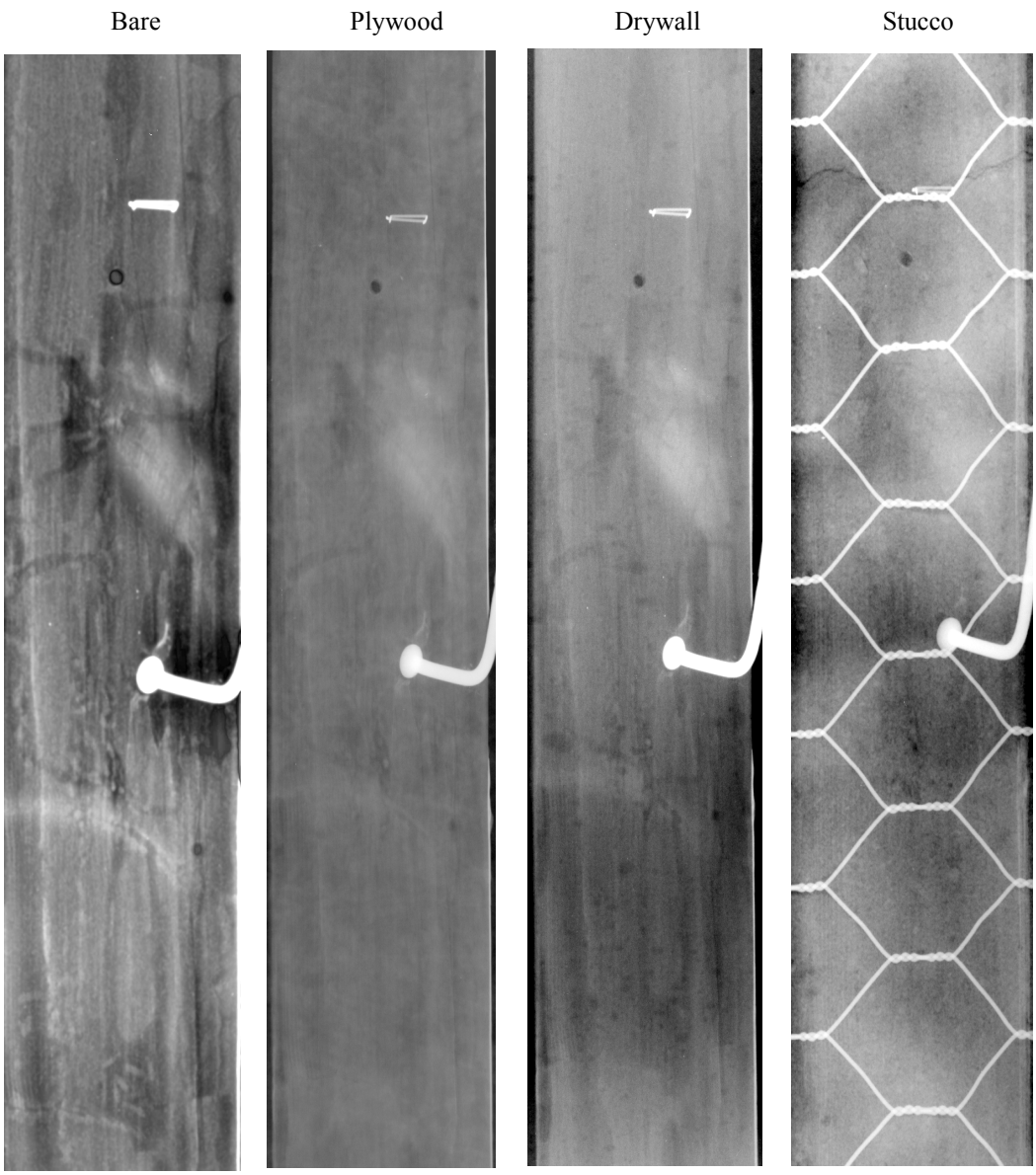

Figure 10. Examples of test board, Active A, containing live drywood termites and galleries viewed with X-ray through wall coverings. These X-rays are the lower 13'' of the board, which showed the most damage. Images were enhanced using the Logos Digital Imaging software then further enhanced with ImageJ such that details could be seen in print version. Coverings included plywood, drywall, and stucco. Test site were stud bays in the Villa Termiti, UC Richmond Field Station, Richmond, CA.

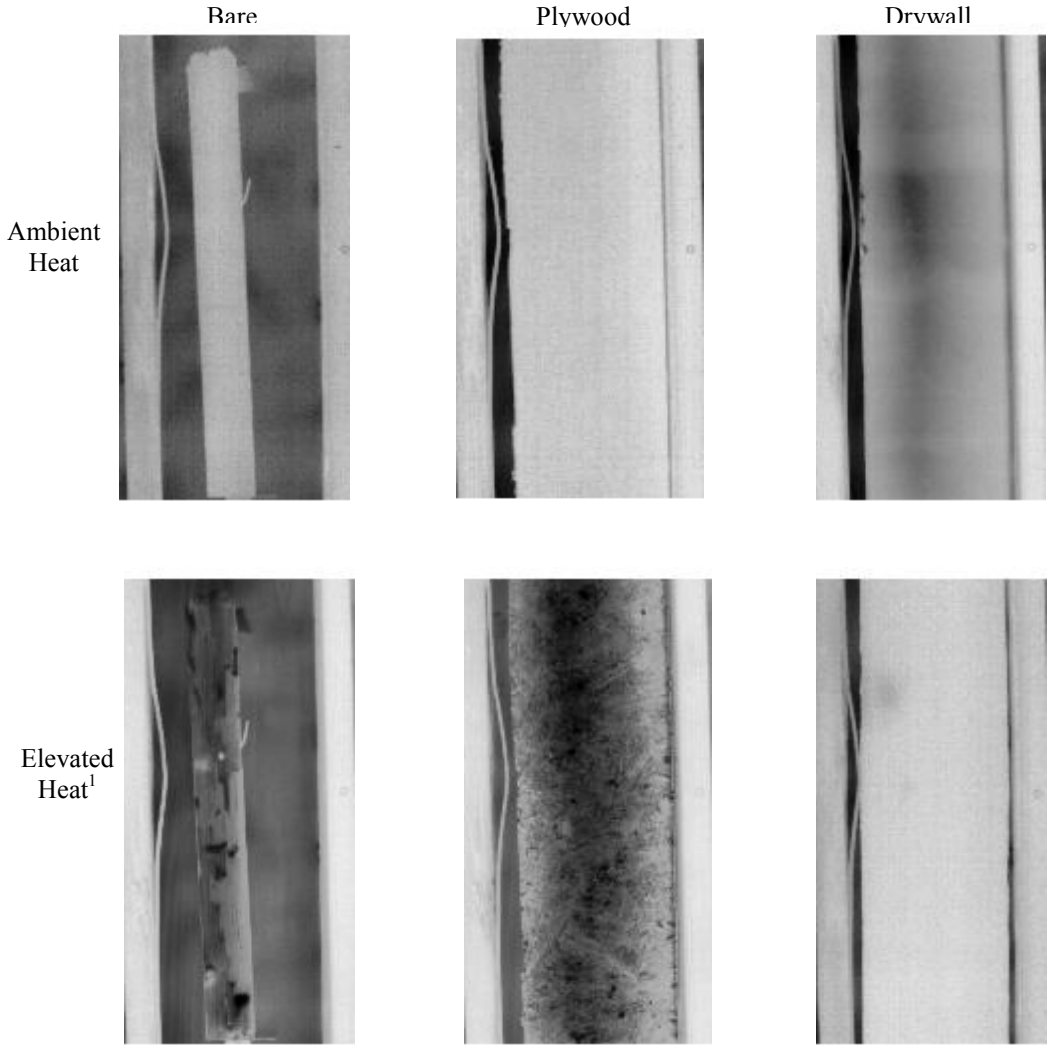

Figure 11. Example of test board, DI A, containing drywood termite damage[2] viewed with an infrared camera through wall coverings. Coverings included plywood and drywall. Test site were stud bays in the Villa Termiti, UC Richmond Field Station, Richmond, CA. [1]Test boards heated at 540 ^{o}C for 10 seconds using a portal heat gun (Heat Gun™ HT 1000, Wagner, Minneapolis, MN). [2]Any patterns seen in the heated board when viewed with infrared correspond to knots, loose paint, or chips in the surface of boards, not as a result of termite damage.

Genetic diversity, colony genetic structure, colony identity and breeding structure of the western drywood termite, *Incisitermes minor* (Hagen).

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Abstract

The western drywood termite, *Incisitermes minor*, represents one of the most important and destructive termite species in the United States. Despite this however, and in comparison to the subterranean termites of the genus *Reticultermes*, nothing is known regarding the genetic colony structure, colony and population differentiation, and breeding structure. Understanding each of these components is essential for the development of, and the understanding of results of management strategies for control. For example, understanding the identity of and the genetic connection, or lack of, between colonies infesting a property will enable the pest control specialist to monitor over time the effectiveness of control strategies given that the molecular methods described here enable accurate identification at the colony level. Thus, using these markers it is possible to track over time change in colony infestion (i.e. if a colony is replaced by a genetically unrelated colony), colony spread (through sampling of individuals at different points in a structure), and colony breeding structure (i.e. the switch from simple families to extended, an important factor linked to the potential for rapid population expansion).

Within this study we have developed a suite of 15 microsatellite markers (small sections of the termite DNA that offer the construction of unique genetic fingerprints for individuals) for *I. minor*. This class of molecular marker is used commonly for studies in other species, including humans, to address questions such as paternity and individual identity (e.g. in crime case). The application of such markers to studies of termite biology and population structure is now

common, however lacking for the drywood termites. Here we applied a selection of these markers to address three key questions relating to infestations in the urban and agricultural environment at several sites across California: 1) Colony identity, 2) Determination of breeding structure, and 3) Colony genetic structure. Results reveal that using these markers we can accurately identify cases of multiple collections actually representing single, potentially expansive infestations by a single colony. Assuming that individuals within these expansive colonies share connections to each other and to the reproductives, such information with prove important in understanding how insecticide is transferred within a colony from a point of introduction. Colony breeding structure proved variable, with all three forms previously observed in the eastern subterranean termites also detected here (i.e. Simple families headed by a single pair of reproductives, Extended families resulting from inbreeding within the colony, and Mixed colonies potentially resulting from the fusion of two or more genetically unrelated colonies). Understanding breeding structure allows us to make predictions of colony size and age, and therefore potentially the level of damage to an infested property. Simple families are likely to be small, capable of producing relatively few workers, thus damage may be limited and restricted to a small area. Extended and mixed families however have significantly greater reproductive output, and therefore colonies may consist of considerable numbers of workers inflicting damage over a larger area within the property. Finally, understanding genetic structure within colonies allows us to understand the levels of inbreeding, an estimate linked closely to both the adaptive ability of a colony, and to the number of secondary reproductives reproducing within a colony. This estimate allows researchers to more accurately predict colony potential size and therefore again may be used to estimate the spatial scale over which damage may occur. All of these factors are especially important in a species, like *I. minor* that is cryptic within properties, thus being able to make

these estimates early in treatment may enable pest operators make more informed decisions of the control strategies available.

Introduction

The western drywood termite, *Incisitermes minor* (Hagen), is recognized as one of the most economically important and destructive termite species in the United States (Su & Scheffrahn 1990). Although native from northwestern Mexico to southwestern United States, due to the ease of both intra- and inter-continental movement of infested furniture and timbers, infestations have been documented outside their native range (Hathorne *et al*. 2000; Xie *et al*. 2001; Indrayani *et al*. 2005). Given the vast economic impact of this species (Rust *et al* 1988), the understanding of population genetic structure and breeding systems is fundamental to the development of more effective management strategies.

I. Identification and characterization of 15 polymorphic microsatellite loci in the western drywood termite, *Incisitermes minor* (Hagen)

Modern molecular markers such as microsatellites, restriction fragment length polymorphisms (RFLP), and mitochondrial DNA sequencing (mtDNA) are now commonly used to address questions relating to species identification, phylogeography, life history, and dispersal behaviors of social insects (Ross 2001, Vargo & Husseneder 2009). Of these, microsatellite markers offer the greatest potential for studies relating to breeding structure and colony identification. According to Avise (2004), microsatellites are probably the most popular and powerful of the current molecular markers. Microsatellite loci are co-dominant markers, with one allele being contributed to the offspring from each of the parents, in a Mendelian fashion. Each microsatellite locus is composed of reiterated short sequences (usually of di, tri, or tetra-nucleotides [see Fig. 1 and 2 for examples]) that are tandemly arrayed at a particular chromosomal location

(Hamada et al. 1984), with variation in repeat copy number often underlying a profusion of distinguished alleles within a population (Avise 2004). Additionally, they can be amplified from small quantities of tissue. The main drawback of these markers is that unless they have already been characterized for the species in question, or even a closely related species, they were, and to many still are, considered difficult, time consuming and costly to isolate. Idrayani et al. (2006) published a set of 10 polymorphic markers for *I. minor*. Of these, however, eight consisted of di-nucleotide repeats, a characteristic often considered unfavorable due to the production of stutter bands when amplified. These stutter bands often make accurate determination of the individual genotype (i.e. homozygote [1 band] or heterozygote [2 bands] difficult to distinguish (See Fig. 1). This is not an issue with tri and tetra-nucleotide loci (See Fig. 2)

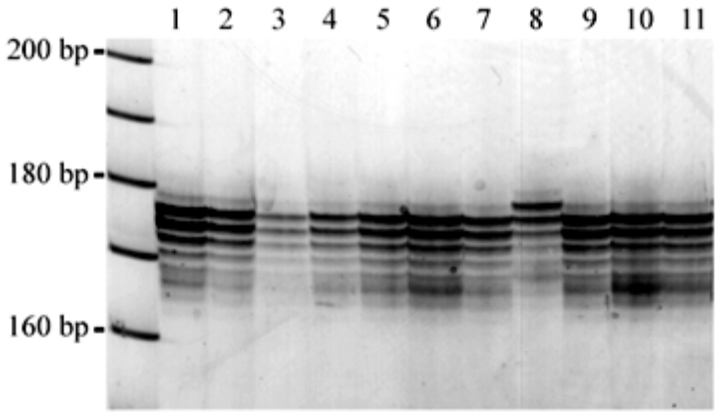

Fig. 1. Example di-nucleotide microsatellite marker. Gel image portrays 11 individuals (1 – 11) and a marker lane (left).

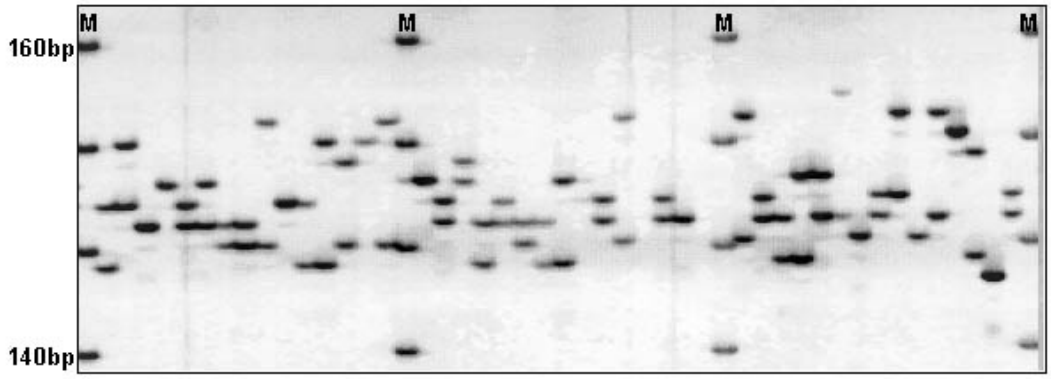

Fig. 2. Example tetra-nucleotide microsatellite marker. Gel image portrays 45 (3 groups of 15) samples and 4 size standard ladders (M).

Given the lack of suitable tri- and tetra-nucleotide microsatellite markers for *I. minor*, we developed an enriched library from which we isolated and characterized 15 polymorphic markers (Booth et al. 2008). Of these, five consisted of tri-nucleotide repeats, eight of tetra-nucleotide repeats, and only two di-nucleotide repeat motifs. Initial screening of 30 individuals, representing six individuals from a total of five geographically separated locations, revealed that the number of unique alleles per microsatellite locus ranged from three to fifteen. Observed heterozygosity, calculated from 23 individuals collected within a single location ranged from 0.050 to 0.866. Preliminary results confirmed these markers yielded sufficient within and between colony/population polymorphism for the resolution of patterns of dispersal, gene flow and colony breeding structure in *I. minor* (Booth et al. 2008).

II. Breeding structure and colony identification

Introduction

Termite colonies are considered to fall into three categories. When reproduction within the colony is controlled by a single king and queen, the colony is referred to as a simple family. Following the death of one or both primary reproductives, the colony may undergo several rounds of inbreeding as a result of reproduction by the secondary neotenics. This is referred to as an extended family. Finally, colonies have been shown to fuse (Deheer & Vargo 2004, 2008; Johns et al. 2009). In these rare events, workers within a colony exhibit genetic ancestry to more than one colony. These are referred to as fused or mixed family colonies.

To date no published data are available regarding the genetic identification of breeding structure, colony identification and colony genetic structure in *I. minor*. An early study by Harvey (1934) described the reproductive life history as follows: Upon king/queen bond formation, and following the excavation of a royal chamber, a queen will lay approximately two to five eggs within the first year, eight to 15 within the second, and fecundity will increase with each successive year until reaching a peak after 10 to 12 years. At five years a colony may consist of the primary reproductives, 20 soldiers and 500 nymphs. A colony at 10 years of age may contain one or more secondary (neotenic) brachypterous reproductives in addition to the primaries. Harvey (1934) acknowledged that these secondary reproductives may supplement egg production, however noted that the existence of more than one pair of functional reproductives, even in older, larger colonies was unlikely. Additional reproductives identified in *I. schwarzi* colonies were considered to result from the fusion of different established colonies (Luykx 1986). In contrast, molecular evidence of breeding structure within the subterranean termites (Rhinotermitidae) [See Vargo and Husseneder (2009) for review], has revealed that secondary reproductives are both common and play a significant role in egg production resulting in extended family breeding structure in this group. Korb & Schneider (2007) report extended families comprised 16% of colonies in the

Australian drywood termite *Cryptotermes secundus* (Hill), while 25% of colonies were the result of fusion events; however no information regarding the details of this study are provided.

The objective of this study was to determine the levels of genetic diversity within geographically separate colonies of *I. minor* using a set of species specific microsatellite markers, thus evaluating the power this marker set offers for future genetic studies of *I. minor*. Within two landscapes (Urban vs. Agricultural) we used these markers to examine the colony genetic structure, identity, and breeding structure.

Materials and Methods

Sample Collection and microsatellite screening

A total of 23 colonies were sampled from 11 distinct collection sites in California. Collection sites were split into two environment classes, urban and agricultural. Ten sites were classified as urban. These were as follows: North Hollywood Hills (n = 5), Oakland (n = 1), Los Angeles (n = 2), Santa Cruz (n = 1), Granada Hills (n = 1), New Port Beach (n = 1), Riverside (n = 2), Lakeview (n = 1), La Jolla (n = 1) and Cypress (n = 1). Agricultural samples were collected within a vineyard near Fresno CA (n = 7). From each distinct collection genomic DNA was extracted from 10 to 20 workers using the DNeasy Tissue Kit (Qiagen, Valencia, CA). These individuals were genotyped at five polymorphic microsatellite loci (*DW*-11, *DW*-12, *DW*-27, *DW*-39, and *DW*-46) following the method described in Booth et al (2008). These loci were selected from the suite of 15 described earlier based on the level of polymorphism and observed heterozygosity.

Colony identity

In order to determine colony identity genotypic differentiation was tested between colony pairs following the permutation methods implemented in FSTAT (Goudet 2001). Additionally log-likelihood (*G*) based exact tests of genotypic differentiation (Goudet et al. 1996) were performed on each pair of sample colonies, as implemented by the program GENEPOP ON THE WEB (option 3, suboption 4; Raymond and Rousset, htpp://wbiomed.curtin.edu/genepop/index.html). Using this program individual locus based estimates of significance were calculated with overall significance determined for each pair-wise comparison after Bonferroni correction based on the number of loci screened. Default parameters within this program were followed.

Classification of Breeding Structure

Colony breeding structure was classified following Vargo (2003), and DeHeer and Vargo (2004). Under this classification colonies are placed into one of three groups. Simple families are those headed by a single pair of primary reproductives. Within these families the genotypes of workers follow those expected for a single pair of parents (i.e. maximum of 4 alleles and no greater than 4 genotypic classes) and if the ratios do not differ significantly from those expected under simple Mendelian patterns of inheritance. The significance of these ratios is determined through a *G*-test performed per locus then summed across all loci. Extended families differ from simple families in the number of reproductives, but like simple families will have no more than four alleles per locus. Under this system the genotypes of the workers are inconsistent with a single pair of reproductives, identified by the presence of more than four genotypic classes at one or more loci, or if the genotypes are consistent with a single pair of reproductives but the *G*-value deviate significantly from those expected under a simple family. Mixed family colonies result from the fusion of two or more colonies and will be evident by greater than four alleles at one or

more loci (See Fig. 3 for a diagrammatic explanation). The power to detect mixed family colonies depends on the variability present in the markers i.e. As detection of a mixed family is dependent on the detection of greater than four alleles at a given locus, loci screened must exhibit 5 or more alleles within the population of interest).

a) Simple Family

Parental genotypes (diploid) – (♂)*AB* x (♀)***C****D*

Possible gametes (haploid egg and sperm) – *A, B, C, D*.

Expected offspring genotypes (predicted using punnett square):

	C	D
A	AC	AD
B	BC	BD

As can be seen there is a maximum of 4 parental alleles (*A,B,C,D*), and therefore no more than 4 genotypic classes (*AC,AD,BC,BD* in this example).

b) Extended Family

Parental genotypes (diploid) – derived from offspring genotypes of simple family (e.g. from example above) Parental (♂1)*AB* x (♀1)***C****D*

Secondary reproductive (♀2)***BD***

Possible gametes (haploid egg and sperm) – *A, B, C, D*.

Expected offspring genotypes (predicted using punnett square):

	B	**C**	**D**
A	AB	AC	AD
B	BB	BC	BD

As can be seen there is a maximum of 4 parental alleles (*A,B,C,D*), and greater than 4 genotypic classes (*AB,BB,AC,AD,BC,BD* in this example).

c) Mixed Family

Parental genotypes (diploid) – Parental (♂1)*AB* x (♀1)***C****D* colony A reproductives

(♂1)*EF* x (♀1)***GH*** colony B reproductives

Possible gametes (haploid egg and sperm) – *A, B, C, D, E, F, G, H*.

Expected offspring genotypes (predicted using punnett square):

	C	**D**	**G**	**H**
A	AC	AD	AG	AH
B	BC	BD	BG	BH

E	EC	ED	EG	EH
F	FC	FD	FG	FH

As can be seen there is greater than 4 parental alleles (*A,B,C,D,E,F,G,H*), and greater than 4 genotypic classes (16 in this example).

Fig. 3. Number and ratio of genotypic classes expected under each potential family structure.

Colony genetic structure

Colony genetic structure was investigated using *F*-statistics following the method of Weir and Cockerham (1984) as implemented in the program FSTAT (Goudet 2001). Analysis was performed over all samples, among urban samples only, and among agricultural samples only. The later two methods were employed so that *F*-values used to infer breeding system were not confounded by higher-level genetic structure. 95% confidence intervals were estimated by bootstrapping over loci with 1000 replications. Those values that did not overlap zero are considered to be significant at the $\alpha = 0.5$ level. The notation of Thorne et al. (1999) and Bulmer et al. (2001) was followed in which each individual colony is considered as a sub-population and the genetic variation is partitioned among the following components: the individual (I), the colony (C), and the total (T). F_{IT} is analogous to the inbreeding coefficient and is a measure of the level of inbreeding in individuals relative to the population. F_{CT} is comparable to the F_{ST} and represents differentiation between colonies. Finally F_{IC} is the coefficient of inbreeding in individuals relative to their colony and is particularly sensitive to the numbers of reproductives present and their mating patterns within colonies. Strongly negative F_{IC} values are expected in simple families, values should approach zero with increasing numbers of reproductives within colonies and be positive with assortative mating among multiple groups of reproductives within colonies or with mixing of adults from different colonies.

Results

Summary statistics

Summary statistics for genetic variability are given in Table 1. Overall an average of 16.67 samples was screened per sample location. Between 12 and 32 alleles were detected per locus, with a mean of 19.2 per locus. Average expected heterozygosity was 0.56 and average observed heterozygosity was 0.58. Samples were then split based on environment class (Urban vs. Agricultural). Within the urban samples, between 11 and 30 alleles were detected per locus, with a mean of 18 per locus. An average of 3.29 alleles was detected within colonies across the five loci. Expected heterozygosity within colonies ranged from 0.30 to 0.71 with an average of 0.57 and observed heterozygosity ranged from 0.29 to 0.78 with an average of 0.61. Within the agricultural samples fewer alleles were detected with between 5 and 9 alleles per locus, and a mean of 7.2 per locus. Across the five loci an average of 2.91 alleles was detected within colonies. Expected heterozygosity within colonies ranged from 0.42 to 0.68 with an average of 0.53 and observed heterozygosity ranged from 0.33 to 0.84 with an average of 0.53. Due to the small sample size within each geographic location estimates of Hardy Weinberg Equilibrium was not calculated.

Colony identity

Given the geographic separation between samples collected in the urban landscape limited samples were likely to display patterns of significant genetic similarity. Based on the results of the permutation tests implemented in FSTAT and the exact *G*-tests implemented in GENEPOP 11 of the 16 collections were considered unique colonies. The North Hollywood Hills collections represented

Table 1. Sample location data and basic summary statistics (number of alleles per locus/sample, mean sample size, mean number of alleles, expected heterozygosity (H_E), and observed heterozygosity (H_O) for 20 colonies of *Incisitermes minor* collected in California, USA. Samples outlines in red and blue represent one colony respectively.

Family type	Population	No. of alleles detected per locus / per sample					Mean sample size	Mean number of alleles	H_E	H_O
		DW-11	*DW*-12	*DW*-27	*DW*-39	*DW*-46				
	Urban									
Simple	Lakeview	1	2	1	4	2	7.4	2	0.3	0.29
	Villa La Jolla	2	4	3	4	2	11.6	3	0.54	0.41
	Vista Real	3	X	2	3	3	18	2.75	0.57	0.78
	North Bend	1	X	3	3	3	16	2.5	0.47	0.53
	Oakland	2	2	2	3	4	20	2.6	0.55	0.68
	Granada Hills	2	3	2	4	X	19.5	2.75	0.48	0.63
	total	1.83	2.75	2.17	3.50	2.80	15.42	2.6	0.49	0.55
Extended	North Hollywood Hills #14	3	3	4	3	3	19.6	3.2	0.64	0.59
	North Hollywood Hills #24	3	3	4	3	3	19.8	3.2	0.67	0.66
	North Hollywood Hills #26	3	3	4	3	3	19.8	3.2	0.63	0.57
	North Hollywood Hills #18	2	4	2	2	4	19.8	2.8	0.56	0.7
	North Hollywood Hills #47	2	4	3	3	4	19.8	2.8	0.52	0.67
	New Port Beach	3	3	3	2	3	20	2.8	0.62	0.55
	total	2.67	3.33	3.33	2.67	3.33	19.80	3.07	0.63	0.57
Mixed	Los Angeles, Board	4	5	5	7	5	19.2	5.2	0.71	0.65
	Los Angeles, Branch	4	4	4	5	6	19.8	4.6	0.69	0.73
	Santa Cruz	2	6	6	7	5	19.8	5.2	0.63	0.58
	Riverside	2	8	3	2	5	17	4	0.51	0.66
	total	3.00	5.75	4.50	5.25	5.25	18.95	4.75	0.63	0.66
Urban mean		2.44	3.86	3.19	3.63	3.67	17.94	3.29	0.57	0.61
Total no. alleles detected		11	30	11	20	18				
	Agricultural									
Simple	B16	2	2	3	3	3	14.8	2.6	0.48	0.55
	B18	1	2	2	3	3	8	2.2	0.42	0.33
	B23	3	X	3	3	2	17.75	2.75	0.57	0.61
		2.00	2.00	2.67	3.00	2.67	13.52	2.52	0.49	0.5
Extended	B6	2	2	3	4	2	13.6	2.6	0.5	0.63
	B12	2	3	3	4	4	13.2	3.2	0.53	0.39
	B15	4	4	2	4	3	12.2	3.4	0.68	0.84
		2.67	3.00	2.67	4.00	3.00	13	3.06	0.57	0.62
Mixed	B5	3	6	2	5	2	16.8	3.6	0.55	0.39
Agricultural mean		2.43	3.17	2.57	3.71	2.71	13.76	2.91	0.53	0.53
Total no. alleles detected		5	8	6	9	8				
	Combined mean	2.43	3.65	3	3.65	3.36	16.67	3.17	0.56	0.58
Total no. alleles detected		12	32	13	20	19				

two distinct colonies. The first colony was comprised of samples #14, 24 and 26, whereas the second colony comprised #'s 18 and 47, both with non-significant *P*-values (Table 2). Put simply, in Table 2, we can see that colonies 14, 24, and 26 share all alleles at each locus. Additionally, the frequency of each genotype produced from these alleles within each colony is found to be comparable to each other. Colonies 18 and 47 share comparable alleles and genotype frequencies at each locus, thus informing us that these two colonies actually represent a single colony. We can see that the colony comprising 14, 24, and 26 is genetically different than the colony comprised of 18 and 47, given the alternate alleles present at each Locus. For example, at locus *DW*-39, colony 14/24/26 possesses alleles 135, 138, and 165, whereas colony 18/47 possesses alleles 150 and 153. Given that these loci are ancestrally different, we can tell instantly that these colonies are genetically different. For further analysis samples #14 and #47 were selected as representative colonies for determination of breeding structure. All colonies sampled within the agricultural landscape were genetically distinct.

Colony breeding structure

Following the detection that the five North Hollywood Hills collection points represented two genetic colonies a total of 20 distinct colonies were available for determination of colony breeding structure (Table one). Of the 13 urban samples, 6 colonies (46.2%) yielded worker genotypes consistent with those expected under a single pair of reproductives. No more than four genotypic classes were detected, all segregating with Mendelian ratios expected for a single pair of reproductives. Three colonies (23.1%), while possessing no more than four alleles per locus, exhibited greater than four genotypic classes (i.e. too many homozygous genotype classes), or Mendelian segregation patterns inconsistent with a single pair of reproductives. These were therefore determined to be

Table 2. Microsatellite alleles and absolute numbers present at five *I. minor* samples collected within the North Hollywood Hills sampling site. Based on allele size and number present colonies 14, 24, and 26 are not genetically different and therefore represent a single colony. Based on the same principle colonies 18 and 47 represent a single colony.

Colony I.D.	Microsatellite locus									
	DW- 11	n	*DW-* 12	n	*DW-* 27	n	*DW-* 39	n	*DW-* 46	n
North Hollywood Hills #14	292	19	205	9	318	9	135	6	144	13
	298	12	241	15	324	10	138	9	162	21
	307	9	260	16	342	10	165	25	168	4
					348	9				
North Hollywood Hills #24	292	20	205	9	318	12	135	11	144	20
	298	9	241	12	324	9	138	11	162	13
	307	11	260	17	342	7	165	18	168	7
					348	12				
North Hollywood Hills #26	292	26	205	7	318	10	135	7	144	17
	298	6	241	11	324	10	138	10	162	17
	307	8	260	20	342	10	165	23	168	6
					348	10				
North Hollywood Hills #18	298	26	245	9	318	9	150	27	148	8
	301	14	264	7	324	31	153	13	158	12
			272	12					168	12
			292	10					172	8
North Hollywood Hills #47	298	28	245	6	318	9	150	32	148	7
	301	12	264	9	324	31	153	8	158	12
			272	11					168	12
			292	14					172	7

extended families. The remaining four colonies (30.77%) exhibited greater than 4 alleles at one or more loci thus providing evidence for colony fusion (mixed colony structure). Within the seven agricultural collections three colonies (42.86%) meet the criteria of being simple families. A further three (42.86%) exhibit an excess of genotypic classes while possessing no more than four alleles

at each locus, and were therefore identified as extended families. The final colony (14.28%) exhibited greater than 4 alleles at more than one locus and was determined to be a mixed family.

Overall, when colonies were combined across habitats 9 (45%) colonies were classed as simple families, 6 (30%) were extended families, and the remaining 5 (25%) were mixed family colonies.

Colony genetic structure

F-statistics and relatedness estimates are shown in Table 2. Overall, workers in both urban and agricultural populations showed signs of inbreeding (F_{IT} = 0.351, 95% C.I. 0.324 – 0.376) with relatedness values of 0.549 (0.507 – 0.597). Population within both environments appeared equally inbred – F_{IT} urban = 0.320 (0.295 – 0.343), F_{IT} agricultural = 0.319 (0.163 – 0.482) (P = 0.49 – paired t-test assuming unequal variances) and had comparable relatedness values (urban = 0.522 [0.471 – 0.588], agricultural = 0.493 [0.420 – 0.567]) (P = 0.28). Across colony type F_{IT} values were not significantly different from each other (P = <0.05). Considering the simple families only, within the urban landscape F_{IC} and r did not differ significantly from those expected from simple families headed by unrelated reproductives. Comparable results were obtained for simple family colonies within the agricultural landscape. Considering extended families, between landscapes with the exception of estimates of r, values for F_{IT}, F_{CT} and F_{IC} were not significantly different than expected (P = <0.05). The high levels of inbreeding and F_{IC} values nearing zero (95% C.I. overlap zero) observed in both landscapes suggest colonies consist of numerous neotenic reproductives (Table 2, B3 through 5), or multiple groups of neotenic reproductives located in spatially separated reproductive centers within the colony.

Table 3. F-statistics and relatedness coefficients for worker nestmates of I. minor from urban and agricultural landscapes within California, USA, and values expected for some possible breeding systems of subterranean termites as derived from computer simulations.

Family type	F_{IT}	F_{CT}	F_{IC}	r
Urban				
Simple (n = 6)	0.308	0.427	-0.208	0.653
(95% C.I)	0.152 - 0.480	0.318 - 0.533	-0.333 to - 0.051	0.539 - 0.738
Extended (n = 3)	0.293	0.22	0.093	0.34
(95% C.I)	0.017 - 0.547	0.151 - 0.283	-0.177 - 0.378	0.270 - 0.399
Mixed (n = 4)	0.285	0.308	-0.033	0.479
(95% C.I)	0.205 - 0.355	0.221 - 0.415	-0.128 - 0.049	0.360 - 0.613
All (n = 13)	0.32	0.344	-0.037	0.522
(95% C.I)	0.295 - 0.343	0.314 - 0.392	-0.111 - 0.025	0.471 - 0.588
Agricultural				
Simple (n = 3)	0.412	0.435	-0.04	0.616
(95% C.I)	0.199 - 0.614	0.307 - 0.551	-0.312 - 0.319	0.442 - 0.753
Extended (n = 3)	0.256	0.308	-0.075	0.491
(95% C.I)	0.021 - 0.464	0.256 - 0.354	-0.321 - 0.190	0.444 - 0.546
Mixed (n = 1)	NA	NA	NA	NA
(95% C.I)				
All (n = 7)	0.319	0.325	-0.009	0.493
(95% C.I)	0.163 - 0.482	0.299 - 0.357	-0.242 - 0.230	0.420 - 0.567
Overall (n = 20)	0.351	0.370	-0.031	0.549
(95% C.I)	0.324 - 0.376	0.344 - 0.408	-0.094 - 0.022	0.507 - 0.597
Simulated breeding system				
(A) Simple-family colonies headed by outbred reproductive pairs	0	0.25	-0.33	0.5
(B) Extended-family colonies with inbreeding among neotenics				
(1) $N_f = N_m = 1$, X = 1	0.33	0.42	-0.14	0.62
(2) $N_f = N_m = 1$, X = 3	0.57	0.65	-0.22	0.82
(3) $N_f = N_m = 10$, X = 1	0.33	0.34	-0.01	0.51
(4) $N_f = N_m = 100$, X = 3	0.34	0.34	0.00	0.51
(5) $N_f = 5$, $N_m = 1$, X = 1	0.27	0.34	-0.11	0.53

This later scenario would be expected where nest budding events have occurred with interconnected daughter nests. Due to the small sample size, and thus large 95% confidence intervals, accurate determination of the number of neotenic reproductives could not be made.

Discussion

Results presented here represent the first documentation of breeding structure, colony identification, and colony genetic structure to date for *I. minor* using highly polymorphic molecular markers. The high level of polymorphism (12 to 32 alleles per locus) exhibited at these five loci proved highly suitable for colony identification, and therefore may represent a powerful tool for future studies investigating colony survival and re-infestation events post insecticide treatment, informing the pest management professional (PMP) of the efficiency of a given treatment.

Our findings provide an insight into the composition of colonies in both the urban and agricultural landscapes. The majority of colonies (45%) are composed of a single pair of reproductives and their worker / soldier progeny. Thus, according to Harvey (1934) these probably represent colonies that are up to or just over 5 years of age. These are colonies that have yet to produce neotenic reproductives or those that contain neotenics that have not yet produced progeny. It is not uncommon to find simple families as the predominant family type within termite populations. For example, Vargo et al. (2006) detected between 48% and approximately 82% of colonies of the Formosan termite, *Coptotermes formosanus*, collected within North Carolina, South Carolina and Louisiana, to be simply families. In a study of colony organization in the subterranean termite *Reticulitermes flavipes*, DeHeer and Vargo (2004) found 70% of colonies to be simple families headed by a single pair of reproductives.

In the drywood termite *I. schwarzi* Luykx (1986) suggested that additional reproductives found within a colony were the result of fusion events with different established colonies. Our results do not support this hypothesis as being consistent with *I. minor*. Across both landscapes extended families composed of multiple reproductive neotenics corresponded to 30% of the colonies sampled. These colonies all exhibited highly positive F_{IT} values and positive or nearing zero F_{IS} values. Accurate estimation of the number of reproductive neotenics within extended families based on the simulations generated by Thorne et al. (1999) and Bulmer et al. (2001) are impossible due to the small sample size and therefore the large confidence intervals. Our results, however, suggest that multiple reproductive neotenics contribute to offspring production in these colonies. If we follow the proposed age structure outlined in Harvey (1934), these extended families represent colonies that are approximately 10 years of age or older, and therefore, structural damage to a building may be extensive.

Mixed family colonies, or colony fusion, as observed in this study may prove difficult to explain. Korb & Schneider (2007) present a record of mixed colonies representing 25% of those sampled, however actual data regarding these are not available. Mixed family colonies have been recorded in a number of termite species, including *R. flavipes* (DeHeer & Vargo 2004), *R. grassei* (Clément 1981; Clément et al. 2001 but not confirmed by DeHeer et al. (2005) using microsatellite markers), *Mastotermes darwiniensis* (Goodisman & Crozier 2002), *Macrotermes michaelseni* (Hacker et al. 2005), and *Zootermopsis nevadensis* (Aldrich & Kambhampati 2007). Explanations as to how these mixed family colonies form have been broadly divided into two categories: those driven by worker foraging behaviors and those driven my alate reproductive strategies. Clément (1981) and Clément et al. (2001) documented genetic and behavioral evidence that suggests a breakdown of nest mate recognition, resulting in colonies associated in close proximity to each other overlapping in foraging area. Whether this results in

eventual reproduction events between reproductives of these adjacent colonies is unlikely given that Pickens (1934) documents the immediate destruction of primary reproductives of *R. hesperus* when introduced into established colonies. Thus in this instance colonies may not actually represent a mixing of genetic lineages within a large colony, but rather an event were colony foraging areas overlap. *M. michaelseni*, in contrast, exhibits true mixed colony composition with the foundation of colonies occurring through pleometrosis. Here, multiple unrelated female alates cooperate during colony formation and contribute to the production of sterile (workers and soldiers) (Hacker et al. 2005). Johns et al. (2009) presented evidence that colony fusion events may occur in the primitive dampwood termites, *Zootermopsis nevadensis*. Here independent colonies may be formed when pairs make use of the same piece of wood. Dampwood termites are single piece nesters; hence it is likely that eventually colony foraging areas within the wood will come into contact. At this time kings and/or queens are killed and the surviving members merge as one colony. Following encounters, members of each colony cooperate as a single social unit and replacement reproductives develop from workers of either or both colonies. Korb & Schneider (2007) describe a situation similar to that of *Z. nevadensis* in the drywood termite, *Cryptotermes secundus*, however both reproductives from one colony are killed leaving only workers from the subordinate colony. These then assist in the duties of the dominant colony. Whether, or not, secondary neotenics from the subordinate colony then mate with reproductives from the dominant colony is unknown. An alternative explanation may be that samples collected that appear genetically as being fused/mixed may actually represent distinct colonies whose activity centers or nests were proximate within the sampled wood but not actually fused. Thus, resulting in the collection of two distinct colonies inadvertently and labeling them as a single collection.

Conclusion

Given the significant economic impact of *I. minor* in the western U.S., the ability to accurately identify colonies, their breeding structure and genetic structure is of fundamental importance for the formulation of effective management strategies. We present the first results generated using highly informative microsatellite markers for this species and demonstrate the power such markers deliver in exploratory analysis of colony structure and life history. These markers may allow the following to be estimated with relative ease:

1) Colony size and age: Simple family structure may inform us that colonies are relatively small, detected in the early stages of infestation. Higher levels of inbreeding and the determination of colonies as being extended informs us that the colonies are likely to be extensive, containing numerous secondary reproductives each producing workers. Thus given the cryptic nature of this species we can now make an informed decision as to the likely extent of damage without extensive physical investigations of a structure.

2) Colony range: The treatment method employed may vary significantly depending on its range. Using these markers we can determine if colonies are contained to small regions of a property, or if they extend throughout a building. Thus, in instances of a single colony ranging throughout a structure, less environmentally intensive insecticide treatment (for example baits) may be developed to transmitted through the nest system from a single treatment point.

3) Treatment efficacy: When a structure is treated with an insecticide, resulting in apparent colony extinction, its efficacy can be determined through genetic testing. Sampling individuals prior to the treatment and post, assuming a structure becomes reinfested, will allow us to determine if the original colony was successfully exterminated and therefore

determine if re-infestation occurred from an outside source, or if a fragment of the original colony survived to re-infest the structure.

These microsatellite markers represent a new powerful tool for future investigations of drywood termite population genetic structure and dynamics. The wealth of information possible through the application of these molecular markers is likely to inform PCO's of the extent of infestations, the connectivity of infestations geographically, and the success of prior treatments, thus allowing informed decisions to be made in regards to their control.

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QUANTITATIVE CHANGES IN HYDROCARBONS IN FECAL PELLETS OF *Incisitermes minor* OVER TIME MAY PREDICT THE ACTIVE OR INACTIVE STATUS OF COLONIES

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Abstract – Hydrocarbon mixtures extracted from fecal pellets of drywood termites are species-specific can be characterized and used to identify the termites responsible for damage, even though termites are no longer present or cannot be easily recovered. For example, in structures infested by drywood termites, it is common to find fecal pellets, but difficult to remove termites from the finished wood in service. Furthermore, until now, when fecal pellets appear after remedial treatment of the wood or the structure, it has been difficult to determine whether the termites in the structure are alive or dead. We examined the hydrocarbon composition of the workers, alates, and soldiers of *Incisitermes minor* (Hagen) (family Kalotermitidae) and fecal pellets of workers. The results presented in this paper reveal that the hydrocarbons for the most part, were similar among workers, alates, soldiers and pellets, and suggest the stability and robustness on using this quantitative method for the describing the chemical ecology associated with this significant structural pest. Similarly, for the fecal pellets that were aged for one week, one month, three months, and one year after collection also showed similarity and robustness in the number of compounds found (73). The hydrocarbons extracted from termites and fecal pellets did not differ qualitatively. However, over time, the hydrocarbons in the fecal pellets changed quantitatively; 26% of the hydrocarbon peaks either significantly increased or decreased in relative abundance. Nineteen of the hydrocarbons had significant slopes over time, and when the sums of the positive and negative slopes were indexed, produced a highly significant linear correlation coefficient ($r^2 = 0.89$). The quantitative differences between hydrocarbons peaks that are increasing or decreasing in abundance can be used to determine the age of the fecal pellets, and thus help determine whether the colony that produced them is active or inactive.

Key Words – Colony age, cuticular hydrocarbons, drywood termites, fecal pellets, frass, Kalotermitidae, Isoptera.

INTRODUCTION

Drywood termites cause significant damage to wood in structures in the United States. Two species, *Incisitermes minor* (Hagen) and *Cryptotermes brevis* (Walker), are responsible for a majority of the damage caused by drywood termites in the United States (Su and Scheffrahn, 1990; Grace, 2009). The economic cost of control and repair of damage is second only to that of subterranean termites (Su and Scheffrahn, 1990). Remedial control of drywood termites in the United States relies primarily on either (1) fumigation of the entire structure with a toxic gas or with heated air, or (2) localized chemical or physical treatments designed to eradicate small, localized colonies (Lewis and Haverty, 1996). Fumigation treatments are likely to kill all of the termites in a structure. Localized treatments can destroy all termites provided the extent of the gallery system of the colony or colonies is accurately delimited and the treatment is delivered to the entire gallery system of the colony.

The size, number, and dispersion of drywood termite colonies in a structure can vary greatly depending on the age of the infestation and the success of preventative or remedial treatments. Drywood termite colonies are also considered single piece nesters, that is, they nest within their food source and do not forage from the nesting site to their food source (Abe, 1987). In fact large, dispersed colonies (or even small colonies) can live in a single piece of wood or in multiple pieces of wood that are joined together (Grace et al., 2009). Further complicating control of drywood termites with localized treatments is the possibility of numerous small, undetected colonies residing in a structure.

The cryptic nature of drywood termites often precludes identification of their activities. They seldom have obvious or visible external signs of their presence in wood. A commonly utilized sign for determining the presence of wood-inhabiting termites is the

associated occurrence of fecal pellets, which are ejected through a "kick hole" in the external surface of wood from the galleries within (Ebeling, 1975). These pellets are often found as conical piles or scattered on horizontal surfaces below infested wood (Figure 1). These hexagonally sided pellets are diagnostic for drywood termites and can be used to distinguish their damage from other wood-destroying insects (Ebeling, 1975; Moore, 1992).

Drywood termites excrete feces in the form of hard, evenly shaped fecal pellets. These fecal pellets contain the same mixture of hydrocarbons as the insects that produced them, albeit in slightly different proportions (Haverty et al., 2005), and because cuticular hydrocarbons are species specific in termites (Page et al., 2002), can be used to identify the termite species by these chemicals in their feces. Rather than simply signaling the general presence of termites or providing a precise diagnosis of the species of termite inhabiting the wood, we wondered whether these pellets could be chemically characterized to determine the active or inactive status of a colony.

Here we report quantification of the hydrocarbons in pellets of *I. minor* aged for up to one year after they were produced and document the changes in proportions of selected hydrocarbons as an indication of the age of the colony producing them, or the length of time since the pellets were ejected from the gallery system.

METHODS AND MATERIALS

Collection of Termites and Preparation of Termite Containment Unit. Groups of drywood termites, *I. minor*, were removed from one naturally infested board (98.89 by 13.34 by 271.78 cm) collected on 19 July 2006 from Lakeview, California and stored at

the University of California Richmond Field Station. The board was cut across the grain into pieces 5 to 8 cm thick and stored at room temperature. A wood chisel was used to separate pieces of wood 0.5 to 1.0 cm thick. Termites were removed from all visible galleries using forceps. Care was taken to minimize trauma or death to termites during the removal process. All remaining, live termites, including soldiers and primary reproductives (alates), were placed in a termite containment unit (TCU).

Fifteen birch tongue depressors were bundled together, and then three equally spaced holes were drilled across the length of the bundle using a 2.78-mm drill bit. Then bamboo skewers were cut 10 to 13 cm in length and inserted into each of the three holes in the bundle of tongue depressors. Spaces 3 to 6 mm were left between each tongue depressor allowing termite access to all tongue depressors. Two sets of skewered tongue depressors were placed into a clean plastic container (17.5 by 12.5 by 6 cm), one on top of the other, such that the tongue depressors on the top layer fit into the gaps created by the tongue depressors on the bottom layer. Tongue depressors were lightly misted with water then 6,662 drywood termites (a mixture of workers, alates, and soldiers) were placed into the TCU (Figure 2). These TCUs, including termites, were maintained in a dark cabinet in the laboratory at ambient conditions prior to collection of fecal pellets. A single group or colony, representing a single location within California, was prepared in this manner.

Pellet Collection and Aging Process. After all TCUs had acclimated to laboratory conditions for several weeks, new holding chambers were prepared (Figure 2). A total of 3,042 live workers were transferred to a new TCU. All wood debris, pellets, dead termites, and damaged tongue depressors were discarded. The termites in the new

holding chambers were maintained in the laboratory at ambient conditions for one week. At the end of one week, all pellets were removed and sub-samples collected and readied for the aging portion of the study. Concurrently, three samples of 20 workers and three samples of 20 alates and one sample of 20 soldiers were also collected for hydrocarbon analysis. Live termites were placed in a 20-ml scintillation vial and frozen until extraction.

Fecal pellets were separated from debris by sequentially sifting them through successively smaller sieves (Haverty et al., 2005). It was important to avoid contamination of the samples with any extrinsic hydrocarbons, thus all substances such as hand lotion, lip balm, Parafilm and waxed paper were kept away from the work area. Gloves were worn when handling pellets and implements that came in contact with pellets were wiped with a paper towel dampened with absolute ethanol to remove any oils or waxes. Pellets were separated from fine debris by removing individual pellets with forceps, or with an aspirator, until a sample weighing approximately 200 mg was obtained.

In developing this protocol we made one estimate and one critical assumption. The *estimate* was that at least 1,000 pellets were needed for each hydrocarbon analysis (based on preliminary analyses of pellets collected from drywood termites maintained our laboratory). The critical *assumption* was based on work conducted by Scheffrahn et al. (1997) using *C. brevis* and *I. snyderi* (Light) from southern Florida. In that study, 40 *I. snyderi* (psudergates or workers) produced 573 pellets in 4 weeks, slightly less than 150 pellets/week or about 3.5 pellets/individual/week. For our study, we assumed that *I. minor* would produce pellets at the same rate. Thus the total number of termites in our

TCU needed to be 3,000 to 5,000 individuals for this study. Therefore, we prepared one TCU to collect the requisite quantity of fecal pellets for this study.

Sub-sampling intervals were divided into four intervals: 1 wk, 1 mo, 3 mo, and 1 yr from the initial collection date. Each of the four intervals was replicated three times. Sub-samples were stored in clean, 20-ml scintillation vials sealed with 1.5 by 1.5 mm mesh screen. The vials were stored in a dark cabinet at the University of California Richmond Field Station at ambient temperature.

Voucher samples of 20 workers and several soldiers of *I. minor* from this study (fresh, i.e. not dried) were preserved in 99% ethanol and deposited in the Essig Museum, University of California at Berkeley (Haverty et al., 2005).

Extraction Procedure and Characterization of Hydrocarbons. The hydrocarbons from workers, alates, soldiers and fecal pellets of *I. minor* were extracted, characterized, and quantified in the same manner as reported in Haverty et al. (2005). Frozen termite workers were thawed and dried at 70°C for approximately 1 hr before extraction. Each sub-sample of fecal pellets or termites were placed in a 20-ml scintillation vial, and immersed in 10 ml of *n*-hexane for 10 min. After extraction, hydrocarbons were separated from other compounds by pipetting the extract through 4 cm of activated Sigma silica gel (70-230 mesh) in Pasteur pipette mini-columns. An additional 5 ml of clean *n*-hexane were dripped through the silica gel. The resulting hydrocarbon extracts were evaporated to dryness under a stream of nitrogen and redissolved in 60 µl of *n*-hexane for gas chromatography-mass spectrometry (GC-MS) analyses. A 3-µl aliquot was injected into the GS-MS.

GC-MS analyses were performed on an Agilent 6890 gas chromatograph equipped

with an Agilent 5973 Mass Selective Detector interfaced with a computer and Agilent Chemstation data analysis software (G1701CA version C.00.00). The GC-MS was equipped with an HP-1MS, fused silica capillary column (30 m X 0.25 mm ID) and was operated in split mode (with a split ratio of 8:1). Helium was the carrier gas. Each mixture was analyzed by a temperature program, from 200ºC to 320ºC at 3ºC/min, with a final hold of 11 min. Electron impact (EI) mass spectra were obtained at 70eV.

All chemicals of interest found were identified by their retention times and mass spectra. Mass spectra of methyl-branched alkanes were interpreted as described by Blomquist et al. (1987) to identify methyl branch locations. Mass spectra of di- and trimethylalkanes were interpreted as described by Page et al. (1990) and Pomonis et al. (1980). Olefins (unsaturated components) were identified by their mass spectra; however, double bond positions were not determined (Haverty et al., 2005).

In the text and tables, we use shorthand nomenclature to identify individual hydrocarbons or mixtures of hydrocarbons. This shorthand uses a descriptor for the location of methyl groups (X-me), the total number of carbons (CXX) in the hydrocarbon component excluding the methyl branch(es), and the number of double bonds following a colon (CXX:Y). Thus, pentacosane becomes *n*-C25; 11-methylnonacosane becomes 11-meC29; 13,17-dimethylhentriacontane becomes 13,17-dimeC31; and heptatriacontatriene becomes C37:3. Hydrocarbons are presented in the tables for each caste and worker fecal pellets in the order of elution on our GC/MS system.

Statistical Analysis. Integration of the total ion chromatogram was performed using the Agilent Chemstation data analysis software. GC-MS peak areas were converted to percentages of the total hydrocarbon fraction. The percentage of the total hydrocarbon

for each hydrocarbon peak (some peaks contain more than one compound or a mixture of positional isomers) was the response variable of interest. A summary of the average relative amounts of each hydrocarbon peak for workers, alates, and soldiers of *I. minor* was prepared. The overall average of each hydrocarbon peak was calculated for the fecal pellets.

The percentage of each hydrocarbon peak for each aging period was transformed to the natural log of the percentage (dependent variable Y) and regressed against the days of aging (independent variable or X). Hydrocarbons with slopes statistically significantly different from 0 ($\alpha = 0.05$) were separated into two groups, those with a significant positive slope and those with a significant negative slope. For each aging period (one wk, one mo, 3 mo, and one yr) an index of age (I_{age}) was created by subtracting the sum of the percentages of the hydrocarbons with a significant negative slope over time from the sum of the percentages of the hydrocarbons with a positive slope over time [$I_{age} = \sum (PEAK_{positive})$ minus $\sum (PEAK_{negative})$ for each aging period]. The I_{age} for each aging period was then regressed against the aging period.

RESULTS AND DISCUSSION

The cuticular hydrocarbons for *I. minor* pseudergates and fecal pellets were previously characterized (Haverty et al., 2000, 2005). In these papers, a detailed discussion of the composition of the cuticular hydrocarbons was presented. The composition of the hydrocarbon mixture for this species and the hydrocarbons from their fecal pellets in this present study are displayed in Figure 3 and summarized in Table 1.

The cuticular hydrocarbon mixtures of most drywood termite species examined thus far (Haverty et al., 1997, 2000, 2005) reflect a general pattern. Cuticular hydrocarbons occur in two distinct groups: early eluting compounds (23 to 29 or 31 carbons in the parent chain) and late-eluting compounds (37 to 45 carbons in the parent chain). The hydrocarbon mixture of *I. minor* is different in that hydrocarbons occur continuously from the early eluting hydrocarbons with 22 carbons in the parent chain to the late eluting hydrocarbons with 45 carbons in the parent chain (Figure 3).

Hydrocarbons from Termites. Normal alkanes, and dimethylalkanes were the predominant classes of hydrocarbons in all castes and the fecal pellets (Table 2) as was previously reported by Haverty et al. (2000, 2005). *n*-Alkanes comprised from 23.12 to 38.39% of the total hydrocarbon. The unsaturated components constituted from 15.21 to 19.03% of the total hydrocarbon. The terminally branched monomethylalkanes amounted to 3.90 to 5.32% of the total hydrocarbon, whereas internally branched monomethylalkanes totaled 7.70 to 9.01%. Dimethylalkanes made up 25.25 to 36.18% of the total hydrocarbon. *I. minor* also made a homologous series of trimethylalkanes representing 8.26 to 9.48% of the total hydrocarbon.

The hydrocarbons of *I. minor* characterized in this study included all of the compounds reported by Haverty et al. (2005), as well as 18 additional hydrocarbons (Table 1). These additional hydrocarbons were not, however particularly abundant, never representing more than 0.85 percent of the total hydrocarbon (the mixture of 12,16-dimeC34 and C35:1 in soldiers), usually much less. These differences are likely due to the concentration of the extracted hydrocarbons, and possibly colony to colony variation. A more concentrated sample allows detection of minor compounds that might not be evident in a less concentrated sample.

Hydrocarbons from Termite Fecal Pellets. In general, the hydrocarbons from whole-body extracts of I. minor were represented qualitatively and quantitatively in extracts of fecal pellets. As with whole-body extracts of I. minor, dimethylalkanes were the predominant class of hydrocarbon followed by olefins and normal alkanes (Table 2). The hydrocarbon mixtures from whole-body extracts and that of fecal pellets were not identical; minor differences did occur. When hydrocarbons were found in the termites, but not in the fecal pellets, they occurred in very small quantities, less than 0.1% of the total hydrocarbon component. This occurred only twice, for n-C30 and 13-meC30 (Table 1). Therefore, the lack of these two hydrocarbons in fecal pellets could simply be a function of the concentration of the extracts.

Changes in Hydrocarbons of Fecal Pellets over Time. In our characterization of the hydrocarbons of fecal pellets of *I. minor* in this study we identified 72 peaks (Table 1). The change in relative abundance for each hydrocarbon peak over the course of a year does not appear, at first glance, to be very dramatic (Figure 4). However, 19 of the 72 hydrocarbon peaks (26%) had a significant change, either positive or negative, over time. This is a much higher number of statistically significant slopes, significantly different from 0, than would be expected by chance alone (5% of 72 peaks would be < 4). When the non-significant hydrocarbons were removed from the mix, we found five hydrocarbon peaks with positive slopes and 14 with negative slopes (Figure 5). The precision of these slopes is somewhat limited with only four time periods and would be improved to have more unique time periods.

We took the 19 hydrocarbon peaks with significant regressions against age and created an index, I_{age}, (see the Statistical Analysis section in the Methods and Materials) by summing the values with positive slopes and subtracting the sum of values with negative slopes. That index for each of the three replications (A, B, and C at days 0, 30, 90, and 365) was plotted against the associated number of days (Figure 6). A line was fit ($R^2 = 0.8879$) to show the variability of the replicates about the fitted line. This high, adjusted R^2 is strongly suggestive that one might be able to predict age given the index.

At this point we do not understand how the chemistry would affect which peaks increase or decrease over the one-yr period. Those that increased are mono-, di- or trimethylalkanes with carbon chains of 31, 32, or 33. Those that decrease are comprised of five of the six classes of hydrocarbons ranging from C26 to C45. Only the terminally branched hydrocarbons are not represented in peaks with negative slopes. It is not clear to us that the hydrocarbons that increase in relative abundance are inherently more stable than those that decrease in relative abundance. In general, those that increased in abundance tended to have higher relative abundances to begin with.

The composition of the hydrocarbon mixture of insects is genetically controlled (Toolson and Kuper-Simbrón, 1989; Kaib et al., 1991; Page et al., 1991; Coyne et al., 1994). This composition can be slightly affected by diet and environmental conditions (Hadley, 1977; Espelie et al., 1994; Chapman et al., 1995; Howard, 1998; Woodrow et al., 2000). Haverty et al. (1996) demonstrated statistically significant seasonal variation

in the quantities of some of the hydrocarbons of *Coptotermes formosanus* Shiraki from Hawaii; these differences were small and were associated with the production of alates. However, the qualitative mix of hydrocarbons has been shown to be amazingly stable for decades among species of cone beetles in the genus *Conophthorus* (Page et al. 1990).

Haverty et al. (2005) opined as to the origin of the hydrocarbons in drywood termite fecal pellets. This could be as simple as contamination from the cuticles of the termites moving within the gallery system strewn with fecal pellets. Three other scenarios are more likely. First, exuviae, injured or dead individuals, and excess members of any caste are commonly eaten (Moore, 1969). The hydrocarbons ingested could pass through the gut undigested, only to be compacted in the fecal pellets. Second, the termites moving and storing fecal pellets could be secreting a mixture of hydrocarbons from the various glands (labral, mandibular, labial or salivary) associated with the mouthparts (Noirot, 1969). Third, and most likely, hydrocarbons are deposited onto the fecal pellets within the rectum during or after the fecal dehydration process. Because the rectum is lined with cuticle (Child, 1934), hydrocarbons are likely transferred to the epicuticular cells of the rectum and then transferred to the surface, or lining, of the rectum.

The motivation for this study was to aid in the post-evaluation success or failure for drywood termite treatments including fumigation and local application methods. With the recent research finding of sulfuryl fluoride (the only fumigant active ingredient registered in California) being reported as a greenhouse gas (Mühle et al. 2009) and the anticipated increase in local treatments, we felt that there would be considerable interest by the industry, regulatory agencies, and consumers for a simple and accurate means to determine how long a “colony/infestation” has been in a structure. We are confident that

changes in the hydrocarbon chemistry over time are a means to this end. Further refinements in the pellet aging process and objectives for future research include: (1) validate these findings for multiple colonies for additional geographic ranges of *I. minor*, (2) validate these findings starting during different times of the year, and finally (3) explore seasonality in pellet production and quality of hydrocarbons in pellets.

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TABLE 1. RELATIVE ABUNDANCE [a] OF HYDROCARBONS FROM WORKERS, ALATES, SOLDIERS, AND FECAL PELLETS OF *Incisitermes minor* (Hagen)

Hydrocarbons	Workers	Alates	Soldiers	Fecal Pellets
2-meC22 [b]	0.06	0.08	0.09	0.11
n-C23	1.30	1.34	1.48	1.35
2-meC23	1.45	1.72	2.11	2.13
3-meC23	1.31	1.38	1.71	2.06
n-C24	0.46	0.43	0.48	0.32
2-meC24 [b]	0.13	0.19	0.19	0.15
3-meC24 [b]	0.08	0.10	0.12	0.11
n-C25	16.35	11.75	12.64	8.65
2-meC25	0.18	0.27	0.24	0.14
3-meC25	0.23	0.36	0.34	0.26
n-C26	2.25	1.17	1.51	0.87
2-meC26 [b]	0.14	0.13	0.14	0.07
n-C27	14.04	6.81	10.05	5.91

2-meC27 [b]	0.13	0.09	0.13	0.06
3-meC27	0.20	0.19	0.25	0.12
n-C28 [b]	0.43	0.17	0.35	0.15
n-C29	3.38	1.27	2.97	1.38
11-meC29 [b]	0.06	0.15	0.10	0.09
9,13-dimeC29	0.70	0.82	0.91	0.63
9,13,17-trimeC29	0.11	0.27	0.16	0.22
n-C30 [b]	0.04	0.07	0.10	---
13-meC30 [b]	0.01	0.08	0.04	---
10,14-dimeC30	0.64	1.45	0.88	0.90
10,14,18-trimeC30	0.18	0.43	0.24	0.28
n-C31 [b]	0.14	0.10	0.20	0.09
15-; 13-; 11-; 9-meC31 [c]	0.36	0.82	0.42	0.49
13,17-; 11,15-dimeC31 [c]	7.45	13.74	8.55	10.67
9,13,17-trimeC31	0.57	1.19	0.72	0.89
7,11,15-trimeC31	0.54	0.99	0.72	0.81
14-; 12-meC32 [c]	0.17	0.33	0.19	0.27
12,16-dimeC32	2.01	3.57	2.22	2.97
10,14,18-; 8,12,16-trimeC32 [c]	0.46	0.80	0.61	0.82
15-; 13-meC33 [c]	0.93	1.57	0.92	1.30
13,17-; 11,15-; 9,13-dimeC33 [c]	4.63	6.87	5.11	7.41
9,13,17-trimeC33	1.30	2.02	1.86	3.47
7,13,17-trimeC33	0.21	0.32	0.32	0.47
C35:3	1.01	2.93	1.64	1.76
C35:2 [c]	2.31	4.46	2.71	3.55
12,16-dimeC34; C35:1 [b,c]	0.68	1.16	0.85	1.40
10,14,18-; 8,12,16-trimeC34 [b,c]	0.28	0.50	0.45	0.89
15-; 13-; 11-meC35 [c]	0.56	0.85	0.67	1.11
13,17-dimeC35	1.27	1.96	1.83	3.95
9,13,17-trimeC35	0.14	0.28	0.27	0.93
C37:3 [c]	1.74	3.33	2.72	3.28

C37:2 [c]	1.67	2.55	2.18	2.90
12,16-dimeC36	0.17	0.34	0.28	0.71
C37:1	0.78	0.76	0.81	0.83
10,14,18-trimeC36; C37:1 [b,c]	0.44	0.35	0.43	0.58
13; 11-meC37 [c]	0.71	0.60	0.77	0.89
13,17-; 11,15-dimeC37 [c]	0.87	1.27	1.25	2.13
9,13,17-trimeC37	0.09	0.13	0.13	0.30
C39:3 [c]	0.09	0.18	0.29	0.49
C39:3; 12-meC38 [c]	0.49	0.56	0.76	1.03
14,18; 12,16-dimeC38 [c]	0.02	0.14	0.14	0.24
C39:1; 10,14,18-trimeC38 [c]	2.73	1.73	2.53	1.74
13-; 11-meC39 [c]	1.75	1.03	1.70	1.26
13,17-; 11,15-dimeC39 [c]	0.90	1.04	1.36	1.44
9,13,17-trimeC39 [b]	0.41	0.17	0.11	0.20
12-; 10-meC40 [c]	0.38	0.20	0.35	0.26
12,16-dimeC40	0.27	0.27	0.34	0.32
C41:1	3.31	1.70	2.79	1.58
13-; 11-meC41 [c]	2.78	1.45	2.59	1.74
13,17-; 11,15-dimeC41 [c]	3.34	2.90	4.00	3.33
9,13,17-trimeC41	0.79	0.30	0.33	0.42
12-; 10-meC42 [c]	0.30	0.15	0.25	0.26
12,16-dimeC42	0.48	0.37	0.52	0.47
C43:1	2.72	1.25	2.27	1.31
15-; 13-meC43 [c]	1.00	0.46	0.87	0.61
13,17-dimeC43	1.99	1.18	1.93	1.68
14,18-dimeC44 [b]	0.12	0.07	0.12	0.13
C45:1 [b]	0.42	0.16	0.33	0.21
13-meC45 [b]	---	---	---	0.04
13,17-dimeC45 [b]	0.37	0.18	0.34	0.43

[a] Percent of total hydrocarbon composition. Compounds are listed in elution order.

[b] These hydrocarbons were not reported for *I. minor* pseudergates or fecal pellets in Haverty et al. (2005).

[c] An isomeric mixture or two or more compounds co-elute in this peak.

TABLE 2. RELATIVE ABUNDANCE [a] OF HYDROCARBON CLASSES FROM WORKERS, ALATES, SOLDIERS, AND FECAL PELLETS OF *Incisitermes minor* (Hagen)

Hydrocarbon Class	Workers	Alates	Soldiers	Fecal Pellets
Normal Alkanes	38.39	23.12	29.77	18.71
Olefins [b]	18.38	21.11	20.31	20.66
Terminally branched methylalkanes	3.90	4.49	5.32	5.19
Internally branched methylalkanes	9.01	7.70	8.88	8.33
Dimethylalkanes	25.24	36.18	29.79	37.40
Trimethylalkanes	5.09	7.40	5.92	9.71

[a] Percent of total hydrocarbon composition.

[b] All peaks with co-eluting olefins and saturated compounds are summarized as olefins.

Figure 1 (A). A conical pile of pellets of *Incisitermes minor* (Hagen).

Figure 1(B). Scattered pellets of *Incisitermes minor* (Hagen) on a horizontal surface.

Figure. 2. Termite containment unit for collecting fecal pellets from *Incisitermes minor* (Hagen) workers.

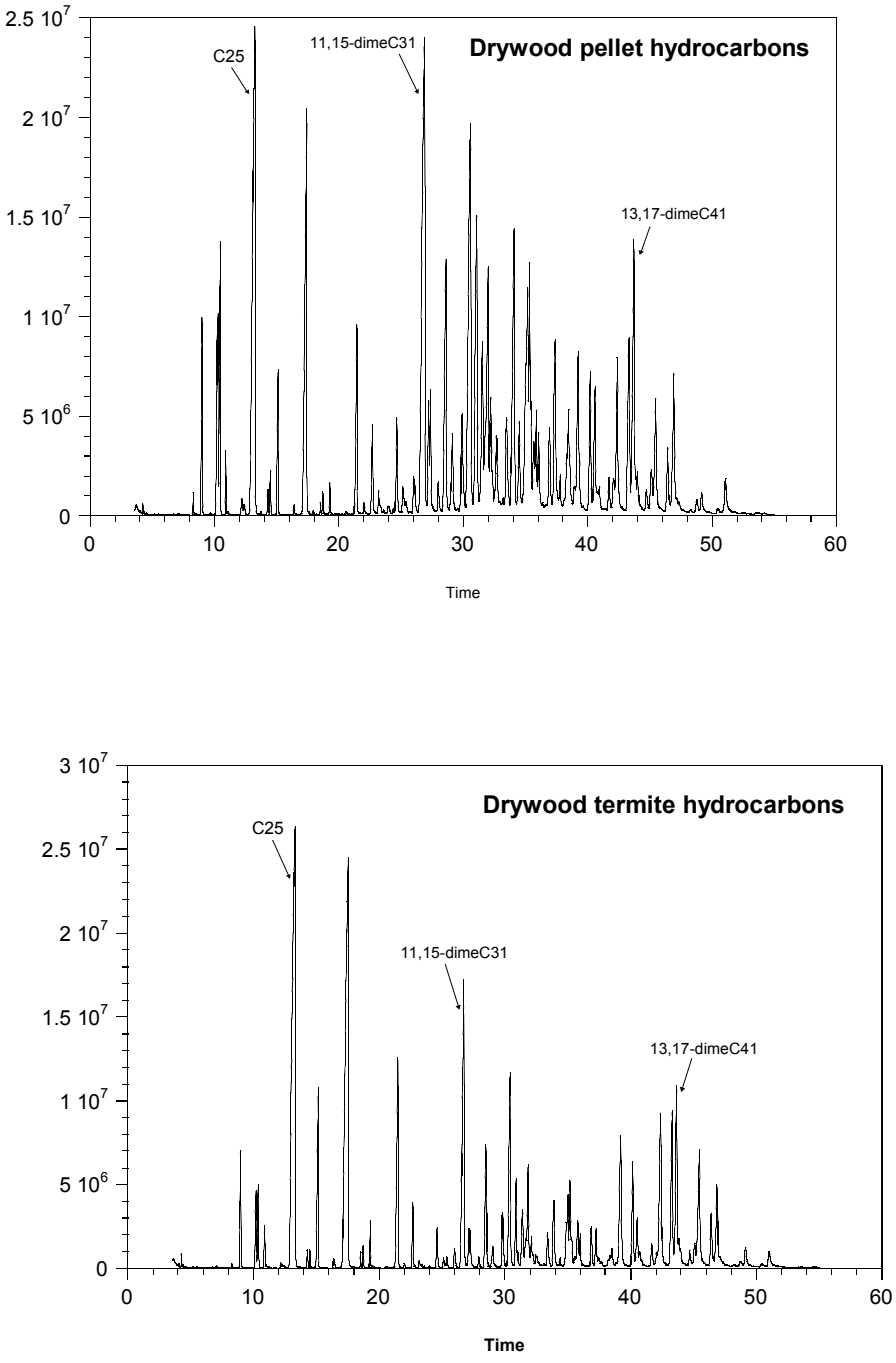

Figure. 3. Total ion chromatogram of cuticular hydrocarbons from workers of *Incisitermes minor* (Hagen) from Lakeview, California, and from fecal pellets from the same collection.

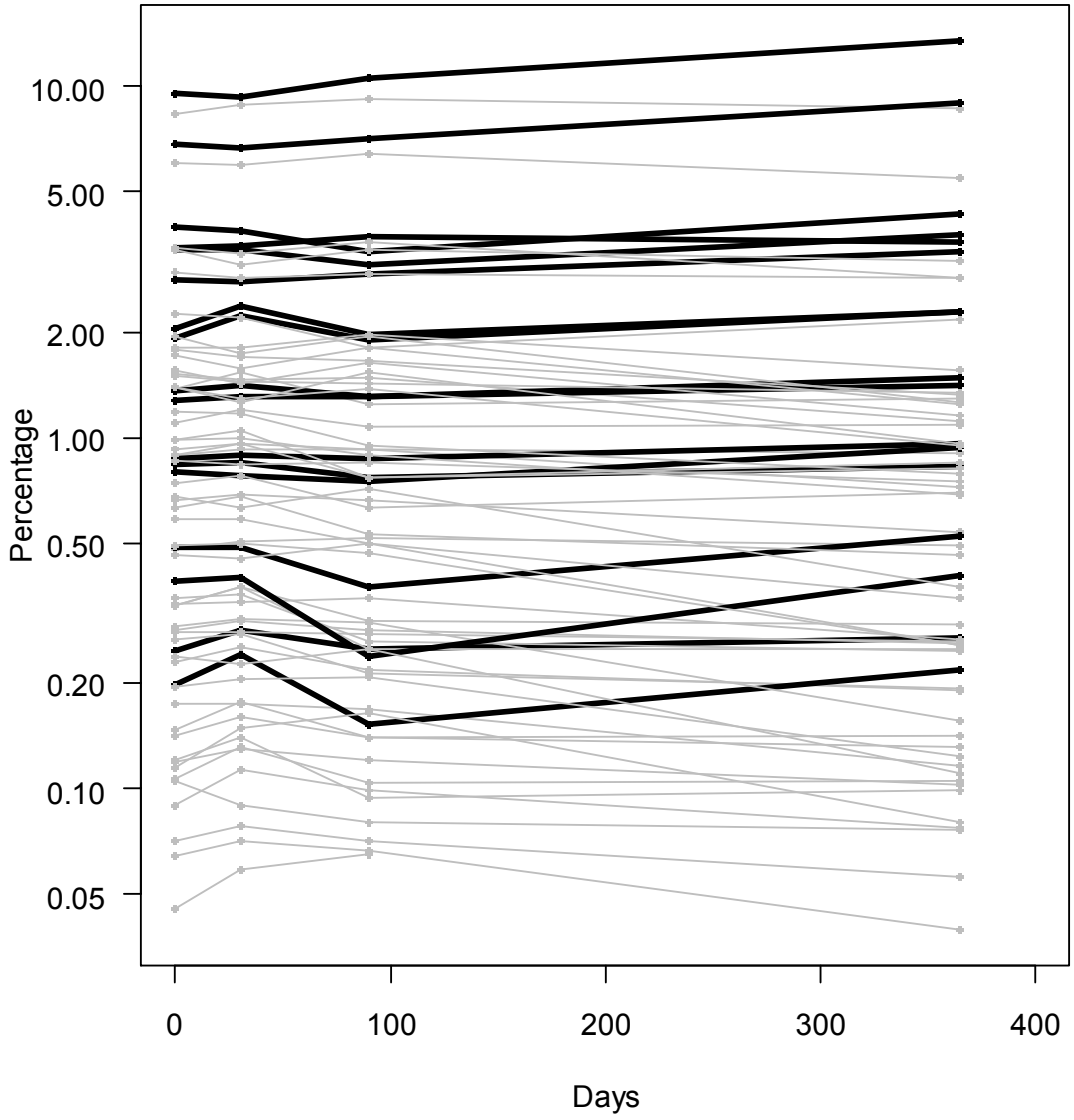

Figure 4. Plots of the log of all hydrocarbon percentages of fecal pellets over time (averaged over the three replicates). A log scaling of the percentages is used to allow more visual separation of the individual hydrocarbon histories.

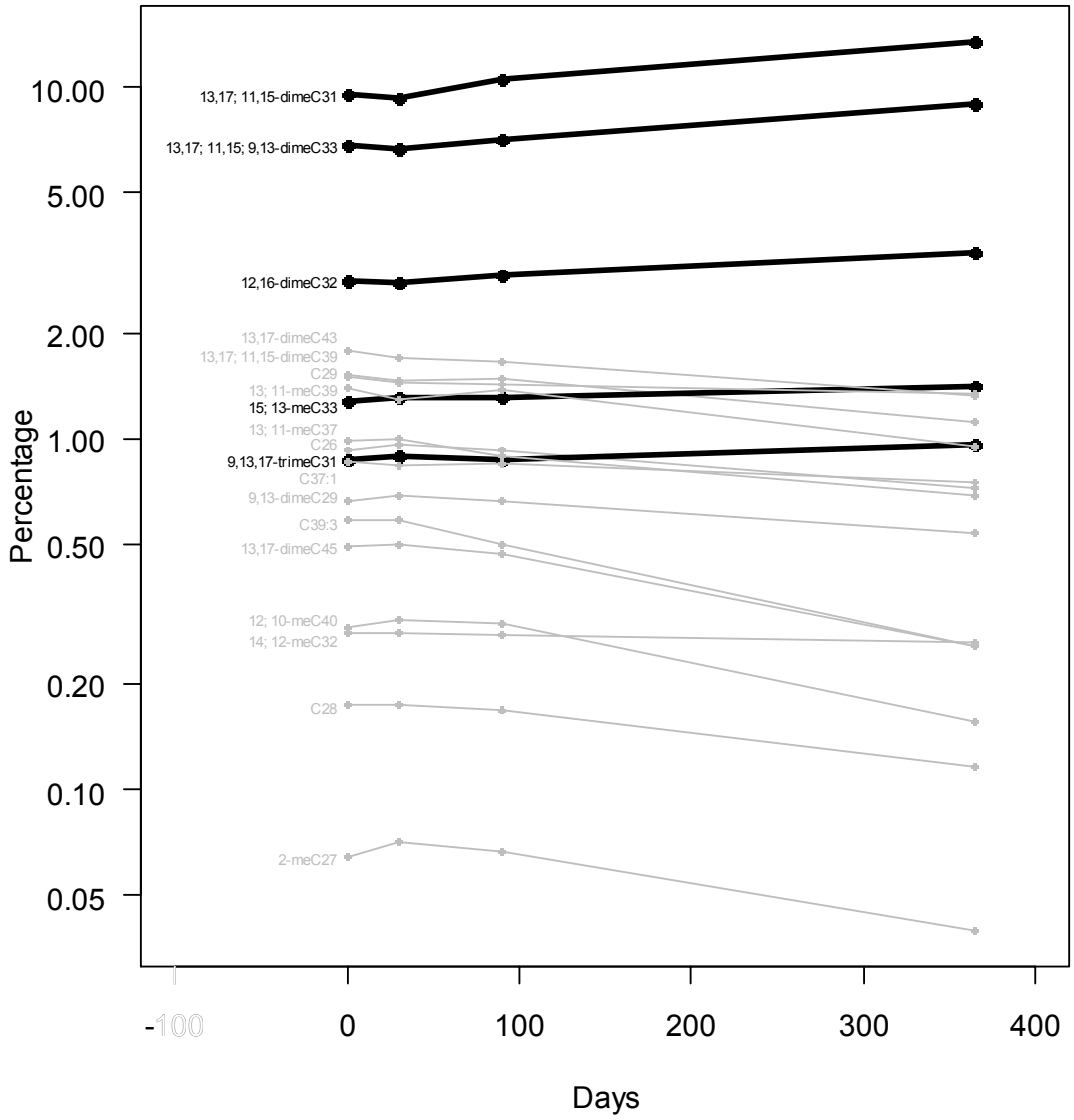

Figure 5. Histories of fecal pellet hydrocarbons with significant slopes over time ($\alpha = 0.05$). Dotted lines indicate a negative slope and dashed lines indicate a positive slope.

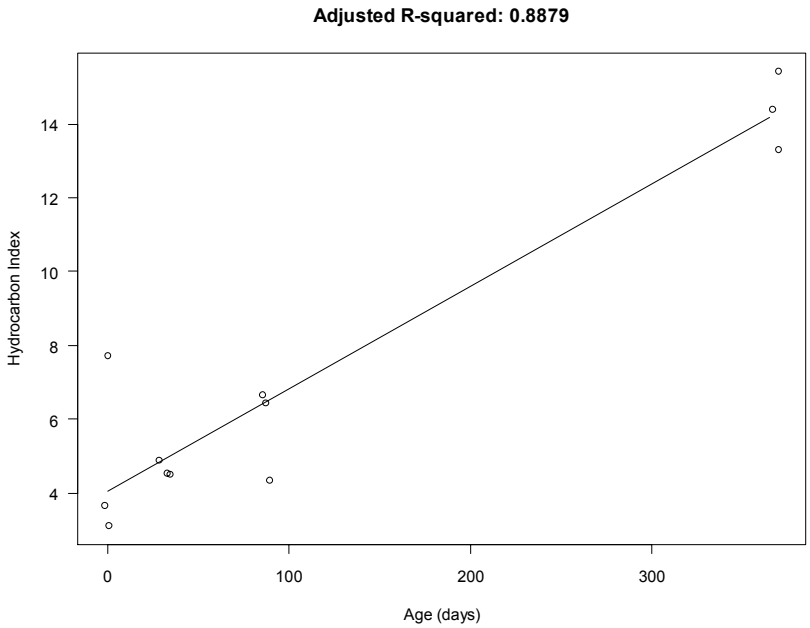

Figure 6. Plot of index of age (I_{age}) against age. All values are jittered a small amount so that there is a bit more separation among points.

DIURNAL AND SEASONAL PATTERNS IN ACTIVITY FOR THE WESTERN DRYWOOD TERMITE, *Incisitermes minor*, FOR NATURALLY INFESTED LOGS

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ABSTRCT

The diurnal and seasonal acoustic emission (AE) activity patterns were measured from five Loquat logs (*Eriobotrya japonica*) containing live Western drywood termite, *Incisitermes minor* (Hagen) infestations. Two additional loquat logs not active with drywood termites were also monitored for acoustic emission activity (un-treated controls). All logs were from the same tree trunk and were similar in size and age. The AE activity was monitored for all seven logs for approximately one year under ambient temperature and humidity conditions in a small wooden structure maintained at the University of California Richmond Field Station, Richmond, California. AE activity for all logs was collected from a custom-designed device that featured seven subsurface sensors each containing a 40 KHz piezoelectric crystal. Termite AE activity was recorded and amplified by using a 40 KHz subsurface sensor with 60 db of gain to amplify the signal. Data from all sensors was randomly collected every three minutes during a 24-hour day for eleven months starting in May 2008. Temperature in ^{o}C and relative humidity (%) was also collected during each AE sampling of data. All logs active with drywood termites displayed a similar diurnal cycle of AE activity that closely followed temperature. Four sensors displayed a single peak of AE activity late in the afternoon (5-6 pm) for each day. A single log displayed a second peak much later in the day near midnight. Seasonally, AE activity was greater during the warmer summer months compared to the cooler winter months was significantly associated with the rise and fall in temperature. Relative humidity did not appear to affect the cyclic nature of AE activity during a 24-hour day. The possible mechanisms that drive the circadian rhythm of AE activity, multiple peaks, and possible implications on what these findings may have on drywood termite natural biology, inspections, and post-treatment are discussed.

Key Words – activity patterns, (diurnal) feeding periodicity, circadian rhythms, seasonality, drywood termites, acoustic emissions, Kalotermitidae, Isoptera.

INTRODUCTION

Much of the success achieved by termites in becoming dominant terrestrial organisms has been attributed to their ability to exploit the rhythmic pattern of the appearance and disappearance of cellulose resources defined by the environmental parameters of sun, humidity, precipitation, and temperature. For subterranean termites, their foraging habits have been well studied especially for desert, Mediterranean, and temperate climates (Haverty et al. 1974; Haverty et al. 1975; La Fage et al. 1976; Esenther 1980; Su and Scheffrahn 1988; Grace et al. 1989; Jones 1990; French 1991; Haagsma and Rust 1995; Forschler and Townsend 1996; Haverty et al. 1999, 2000; Baker and Haverty 2007). Using various topical and in-ground monitors, temperature probes and precipitation amounts, foraging and wood consumption was found to be seasonally highly rhythmic and closely followed temperature; highest when warm versus cold, (Haverty et a. 1974, La Fage et al. 1976). Haverty et al. 1999) or associated with rainfall amounts (Haverty et al. 1974, La Fage et al. 1976). These foraging and consumption patterns have been coined circadian rhythms (Schotland and Sehgal 2001) and are common across many phyla of plants, microbes, and animals on earth. However, for drywood termites (Kalotermitidae), because they are single piece nesters and live deep in wood, there are no reports of topical or in-ground monitors and would be irrelevant because their nests and foraging are not soil based. Acoustic emission is another

technology that could be exploited to explore the diurnal and seasonal patterns in drywood termite foraging and feeding activity.

Acoustic emission (AE). Many genera and families of termites produce vibrations in wood while feeding and by alarm calls from the head banging of soldiers and body vibrations of workers. Some of these vibrations can be heard by the human ear or when sounds are amplified by microphone (Emerson and Simpson 1929, Pence et al. 1954, Wilson 1971, Stuart 1988, Kirchner et al. 1994). Several genera in the Kalotermitidae also produce audible sounds (Emerson and Simpson 1929). These sounds are produced during feeding (Fujii et al. 1990, Imamura et al. 1991, Scheffrahn et al. 1993, Fujii et al. 1995, Matsuoka et al. 1996) and by vibratory movements of workers (Lewis et al. 1991, Beall et al. 1991, Leis et al. 1992, Maistrello and Sbrenna 1996, Dunegan 2001). More recently, researchers from Australia reported the use of vibrational signals from worker drywood termites (*Cryptotermes domesticus* Authority? and *C. secundus* Authority) to differentiate wood food source and to assess wood resource size (Evans et al. 2004, 2007; and Ra Inta et al. 2007). Surface and subsurface probes are available for AE devices and the successful detection of drywood termites in laboratory settings is at least 80% (Lewis and Lemaster 1991, Lewis et al. 1991, Scheffrahn et al. 1993, 1997, Lewis and Haverty 1996, Lemaster et al. 1997, Lewis et al. 2004, 2005). Wall coverings that include drywall and paneling can impede sensor and AE performance. Distance in detection has been reported from 80 cm to 240 cm along the length of a board and < 8 cm across the grain (Scheffrahn et al. 1993, Lemaster et al. 1997).

Drywood termite seasonal activities. Seasonal activity patterns of drywood termites are very important for their detection and treatment. A common seasonally activity for drywood termites is swarming. In California, drywood termites annually swarm during the day starting in summer and continuing into fall (Ebeling 1978). Feeding and foraging is another drywood termite activity; however, little is known when it occurs. The cryptic and hidden behavior of drywood termites deep inside wood hinders studies that explore their normal feeding and foraging behavior. Using AE technology to record *Incisitermes minor* (Hagen) feeding, Lemaster et al. (1997) found no periodicity in feeding in a 24-hour day. However, the investigation only ran for 1 week. Researchers at Kyoto University also used AE to monitor *I. minor* feeding when affected by different laboratory temperatures and relative humidity (%); however, they did not report diurnal or seasonal AE cyclical activity (Indrayani et al. 2000). Another investigation using AE monitoring of local chemical treatments from field studies in southern California, found *I. minor* infestations in untreated locations of structures declined in activity during winter months (V. Lewis, unpublished data). Since this study involved only four post-treatment inspection dates, it is impossible to make definitive statements on seasonal foraging of drywood termites.

Little research has been conducted on the movement patterns of drywood termites. Currently, only the speed of locomotion of *I. minor* (1.4 cm/s) in response to temperature and light is known (Cabrera and Rust 1996, 2000). Although, drywood termite (*I.*

fruticavus) movement was inferred from studies that measured daily changes in temperature inside galleries for the Jojoba shrub, *Simmondsia chinensis* (Link) (Rust et al. 1979), the seasonality of movement for drywood termite species within structures for California remains poorly understood. The purpose for our study was to explore for diurnal or seasonal patterns of drywood termites feeding and vibrational activity from naturally infested logs. The applications of this research include improved inspections by knowing the best times of the day and year when drywood termites are most active, future natural history investigations, and post-treatment evaluations of remedial treatments. What about pretreatment evaluations? You could miss an active infestation also.

METHODS AND MATERIALS

Naturally infested logs preparations. Ninety logs from a large Loquat tree (*Eriobotrya japonica* (Thunb.) Lindl.), were collected during the summer of 2007 from a private residence in southern California (Granada Hills, CA) and brought to the laboratory for further processing. The logs were similar in maximum diameter (average 15.1 cm, range 11.4 cm to 19.9), length (average 47.3 cm, range 46.1 cm to 49.0 cm), and age (the homeowner stated the tree was mature, at least 4.5 m in height when cut for firewood in 1998). All logs revealed visual signs of drywood termite activity (damaged wood that cut across spring and summer growth rings and presence of six-sided frass pellets). After visual searches, the logs were stored at the University of California Richmond Field Station (RFS) in the Villa Termiti (Lewis and Haverty 1996) at ambient □

temperature and humidity. To verify candidate logs were active with drywood termites, using the methods from Lewis and Haverty 1996, three 1-min recordings from their centers along the top long side were taken. All boards were searched for AE activity using a hand-held device (Tracker, Dunegan Engineering, Midland, TX) (Fig. 1). The senor portion of this device contains a piezoelectric crystal of resonant frequency 40 kHz signal with a sensitivity of 1 v/m, amplified by 60 db of gain, and transducer sensitivity of 200 mV (Dunegan 2005). The sensor waveguide (2.8 mm in dia) is for subsurface use and was inserted 1.2 cm into the wood surface. The device displays a digital number in its LCD screen that represents termite activity as AE events (Fig. 1). An event is the actual pulling of wood fibers by termites, although some movements through galleries may produce additional AE activity. Ring down count is the propagation of AE-generated feeding energy throughout the board. A homologues example of these variables can be thought of as a raindrop hitting a pond (event) and similarly the wave propagation amplitude throughout the pond being AE ring down count. AE events and ring down counts are good measures of the presence or absence of termites (80%); however they are only moderately associated with the number of termites within infested wood at the time of recording, $r^2 = 45\%$ (Scheffrahn et al. 1993). There are at least three commercial AE termite activity devices available in North America to assist with termite inspections and searches (Lewis, unpublished data).

Multi-channel AE monitoring equipment. All logs having at least 300 counts per minute, averaged over three one-minute recordings and similar in maximum cross-

sectional diameter and length, were chosen to be included in the study. From those 17 logs determined to have high AE activity, seven were randomly chosen. Five were logs used to record AE activity and two logs containing termites were chosen as controls (containing no termites) to measure background AE activity from the surroundings. For the control logs, they were put into an oven (Isotemp model 655F, Fisher Scientific, Pittsburgh, PA) at 105 °C for three days to kill any termites within the logs. All seven logs had a subsurface sensor installed into their long center by drilling a 2.4 mm diameter hole and inserting the sensor probe 1.2 cm deep into wood (Fig. 2). All sensor assignments were chosen randomly. The 3-m long cable from the last of the seven sensors was connected into a port in the back of an AE smart device (Dunegan 2005) and dedicated computer (Dell Corporation, Austin, TX) that stored all data (Fig. 4). All logs were placed onto a small wooden table (152.7 cm by 86 cm by 78 cm) and the AE Smart, dedicated computer, and backup power supply were placed on a second wooden desk (150 cm by 74 cm by 75 cm) near the first table (Fig. 4). To prevent ants (Argentine ants, *Linpethemia humilie*) (Mayr) from attacking the logs on the table, the legs of the table were placed into small plastic saucers (15 cm dia by 3 cm deep) containing a 1 cm deep band of motor oil (SAE 30, Ace Hardware Corporation, Oak Brook, IL). Additional ant prevention measures included applying a 15 cm long band of petroleum jelly (Long Drugs, Walnut Creek, CA) to the cable end leading into the back of main AE Smart controller box. The entire system was visited at least weekly to check on the operating status of the entire system. All data was downloaded onto high capacity (256 MB) portable storage device (Kingston Data Traveler, Fountain Valley, CA) for long-term □

storage and future analysis. Twenty, 3-minute recordings were recorded randomly among the seven sensors for each 60 minute period during the study. In addition, temperature and humidity data (Omega Engineering, Stamford, CT) was recorded for each 3-minute recording and also stored and saved to an electronic spread sheet (Excel Corporation, Lubbock, TX). A backup battery power supply (Back-ups, APC Corporate, W. Kingston, RI) was also installed in the event of unexpected power outages. The entire AE gathering and stored system was run twenty-fours a day for almost one year (June 2008 to May 2009).

AE equipment storage and housing. All logs, AE and temperature equipment were stored in a small wooden building at the University of California Richmond Field Station, Richmond, CA. The building was 8.2 m by 4.9 m, 40.2 m^2, one story in height, and had a slab foundation (Fig. 4). It was approximately 50 years old. The construction type was double wall with Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco V.) studs, redwood interior wall and ceiling paneling. The exterior was wood ply board. The building had five windows for natural light. Three windows were on the northeast side (91.4 cm by 60.9 cm in size) and two on the southeast side of the building (121.9 cm by 111.8 cm and 122 cm by 91.4 cm in size). There was no insulation, air conditioning or heaters in the building, just ambient environmental conditions. The roof was tar and shingle and was in good condition. To monitor human traffic into the building, a sign up log system sheet of paper was installed to note the date, time, and individuals who entered the building and a warning sign was posted on the exterior door entry to

notified individuals before going in that a vibration sensitive research project was being conducted.

Statistical analysis. The averages for AE activity, temperature, and relative humidity were plotted for each sensor per hour per day for active and inactive logs.

RESULTS

Diurnal patterns in AE activity. Within a 24-hour day, AE activity (both event and ring down count) among the logs displayed a non-linear pattern of activity (Figs. 5 and 6). The pattern of AE activity data was sinusoidal in shape and statistically significant for all sensors. AE activity was lowest during the morning, increased in the afternoon, and peaked in late afternoon (6 pm), then declined until mid-morning. For log (#1), a second peak of AE activity (events and ring down counts) was recorded late into the evening at midnight and was also statistically significant. From the AE activity sensor results (y-axis), the infestations could be categorized into three groups; sensor 3 represented the smallest, sensors 1, 4, and 5 were roughly three times the size of sensor 3, and senor 2 was the largest, almost seven times the size of sensor 3. Temperature was correlated with the rise and fall in AE activity; warmer temperatures were associated with increasing activity (Figs. 5 and 6). However, relative humidity was not statistically correlated with AE activity.

Seasonal patterns in AE activity. Seasonally, AE activity both events and ring down counts displayed a non-linear pattern of increasing and decreasing values associated with temperature (Figs. 7 and 8). The relationship between seasonal AE activity and month during the year was statistically significant. AE activity was highest during the warmer spring and summer months compared to winter. However, an increase in daytime temperature or a sudden heat wave, even in January and February 2009, resulted in an increased burst of AE activity (Figs. 7, 8, and 9). Seasonally, there was a statistically significant association between temperature and AE activity. Humidity did not have a statistically significant (Fig. 9) impact on AE activity during the 11-month study.

Using all data from all dates and times of the day a regression model was fit for each sensor to predict AE activity, both events and ring down counts. The residuals of both AE counts and TTL counts were closer to being normally distributed with a constant variance when the square root of the counts was used as the dependent variable. All possible polynomials of order two for temperature and relative humidity were examined but a simpler model of the main effects of temperature and relative humidity plus a temperature/relative humidity interaction term performed nearly as well. The model is below and includes; a = actual event counts, T = temperature in ^{o}C at the time of AE recording, and RH = relative humidity (%) at the time of AE recording.

$$\sqrt{Count} = a_0 + a_1 \cdot T + a_2 \cdot RH + a_3 \cdot T \cdot RH + error$$

The estimates of the coefficients for predicting the square root of the AE events and ring down counts are presented in Table 1. Sensors 1 through 3 all had statistically significant slopes for temperature, relative humidity and intercept. Sensor 4 did not have a statistically significant intercept and Sensor 5 did not have a statistically significant association with temperature or relative humidity. The correlation coefficients for the regression model in predicting AE event and ring down count are presented in Table 2. The total variance described by the regression model for AE events and ring down counts ranged from 62.4 % to 71.9% for sensors 1 to 4. The describe variance for Sensor 5 was considerably lower at 13.9% for events and 28.8% for ring down count compared to the other sensors.

DISCUSSION

There are few papers that present diurnal or seasonal AE activity data for *I. minor*. Using 100 workers contained in an artificially infested wooden block held at constant temperature and humidity, Lemaster et al. 1991 reported AE events results from a single sensor during seven days of testing for *I. minor*. There was no statistical significant cycling or periodicity in AE activity found. The plot of AE activity appeared flat and hovered between 100 to 200 events per hour. No ring down counts data were reported. These authors' maximum AE event results (y-axis maximum) compared favorably with the maximum events reported for sensors 1, 2, 4, and 4 for the current study (Fig. 6). □

There is considerable variance in the size and number of drywood termites dissected and reported from naturally infested logs and structural wood and ranges from 7 to 2,943 (Harvey 1934, Scheffrahn et al. 1993, Lewis and Haverty 1996a, Lewis and Power 2004, Lewis et al. 2005). Obviously, larger colonies and infestation produce greater AE activity. And also clear evident that when drywood termites are allowed to search and forage for wood naturally under ambient conditions, their activity follows a cyclic pattern common to many terrestrial animals.

The second peak of termite activity found for one during a 24-hr day is curious and needs further explanation. The first peak can be explained with raising temperature necessary to heat up the termite physiological mechanisms for locomotion, feeding, and digestion. Perhaps the second peak for some this log has to do with the caste porportions and numbers of the colony, division of labor the workforce (Crosland et al. 1996), or behavior of castes and assigned task (Abushama and Houty 1988, Maistrello and Sbrenna 1998, Hinze and Leuthold 1999). Since we don't know the age of the infestations, it could be different aged groupings of termites within logs varied in number of individuals and behaviors, and leading to the different AE patterns and peaks seen. The gut fauna found in all termites could also be partial responsible for the two AE peaks seen, the first attributed to the combination of temperature and digestive enzymes from the termites themselves, the second peak attributed to the nutritional needs of the gut fauna (Cabrera and Rust 1994).

A second AE activity study was conducted by Indrayani et al. 2006. For this laboratory study *I. minor* workers were also used (10) in small wooden blocks to test the affects of varying temperature and humidity. The tests were of short duration, 12 hr. This study reported that the optimum temperature for peak AE activity was 30° C. Reviewing the temperature and data for the current study, AE activity increased with temperature, even during warm days during seasonally cold months (November 2008 to February 2009; Figs. 4 and 5). Field observations reported to UC Berkeley over the years included complaints of seeing drywood termite pellets in December from a large condominium site in Marina del Rey, CA. Before this study, those pellets were assumed to be background noise and not to be worried about when they occur during colder of winter months. However, with these new findings perhaps the turning on heaters to warm the interiors of apartments and residence during the winter months simulated drywood termite feeding activity.

Knowledge on optimal times for drywood termite foraging could be important to termite inspections. Two species, *Incisitermes minor* (Hagen) and *Cryptotermes brevis* (Walker), are responsible for a majority of the damage caused by drywood termites in the United States (Light 1934, Su and Scheffrahn, 1990, Grace, 2009). The economic cost of control and repair of damage is second only to that of subterranean termites (Su and Scheffrahn, 1990). Traditional inspections are visual and based on visual searches for damaged wood or pellets. The data from this study suggests searches for pellets could be enhance by heating the wood to at least 25° C prior to inspection to simulate foraging

and feeding, even in winter. Understanding the underlying mechanism that controls the cyclic pattern, called Zeitgeber (Saunders 2001), will require additional study that include the exclusion of natural light, running additional tests for naturally infested logs at constant temperature and humidity, and modifying the AE system collection hardware and software to sort out locomotion from feeding AE activity.

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Figure 1. A hand-held device used to detect acoustic emission (AE) activity in naturally infested logs. Displayed in the center of the figure is the main component (23.1 cm by 12.7 cm), and subsurface sensor (21.1 cm long and 2.8 cm in dia) is on the left. On the top left of figure is a LCD screen that displays a digital whole number that represents AE termite activity. Manufacturer of Termite Tracker is Dunegan Engineering, Midland, TX.

Figure 2. Seven logs from a large Loquat tree (*Eriobotrya japonica* (Thunb.) Lindl.) used for the study collected during the summer of 2007 from a private residence in southern California (Granada Hills, CA). The logs were similar in maximum diameter (average 15.1 cm, range 11.4 cm to 19.9), length (average 47.3 cm, range 46.1 cm to 49.0 cm), and age (the homeowner stated the tree was mature, at least 4.5 m in height when cut for firewood in 1998). All logs were placed onto a small wooden table (152.7 cm by 86 cm by 78 cm). To prevent ants (Argentine ant, *Linepithema humile* (Mayr)) from attacking the logs on the table, the legs of the table were placed into small plastic saucers (15 cm dia by 3 cm deep) containing a 1 cm deep band of motor oil (SAE 30, Ace Hardware Corporation, Oak Brook, IL). Additional ant prevention measures included a applying a 15 cm long band of petroleum jelly (Long Drugs, Walnut Creek, CA) to the cable end leading into the back of main AE Smart controller box. The seven small white boxes (8.9 cm by 3.5 cm by 3.1 cm) seen in the center of the photograph are AE Smart Max-modules (Dunegan Engineering, Midland, TX) and are used in conjunction with software to control the order of sensor selection (randomly) for AE activity during data collection.

Figure 3. The AE Smart (Dunegan Engineering, Midland, TX), monitor, dedicated computer (Dell Corporation, Austin, TX), and were placed on a second wooden desk near the first table (Fig. 2). The 3-m long cable from the last of the seven sensors was connected into a port in the back of an AE smart device. Seven, 3-minute recordings were recorded randomly among the seven sensors for each 60 minute period during the study. In addition, temperature and humidity data (Omega Engineering, Stamford, CT) was recorded for each 3-minute recording and also stored and saved to an electronic spread sheet (Excel Corporation, Lubbock, TX). A backup battery power supply (Back-ups, APC Corporate, W. Kingston, RI) is also shown in the figure and was also installed in the event of unexpected power outages. The entire AE gathering and stored system was run 24/7 for almost one year (June 2008 to May 2009).

Figure 4. All logs, AE and temperature equipment were stored in a small wooden building at the University of California Richmond Field Station, Richmond, CA. The building was 8.2 m by 4.9 m, 40.2 m^2, one story in height, and had a slab foundation. It was approximately 50 years old. The construction type was double wall with Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco V.) studs, redwood interior wall and ceiling paneling. The exterior was wood ply board. The building had five windows for natural light. Three windows were on the northeast side (91.4 cm by 60.9 cm in size) and two on the southeast side of the building (121.9 by 111.8 and 122 cm by 91.4 cm in size). There were no air conditioning or heaters in the building, just ambient environmental conditions. The roof was tar and shingle and was in good condition.

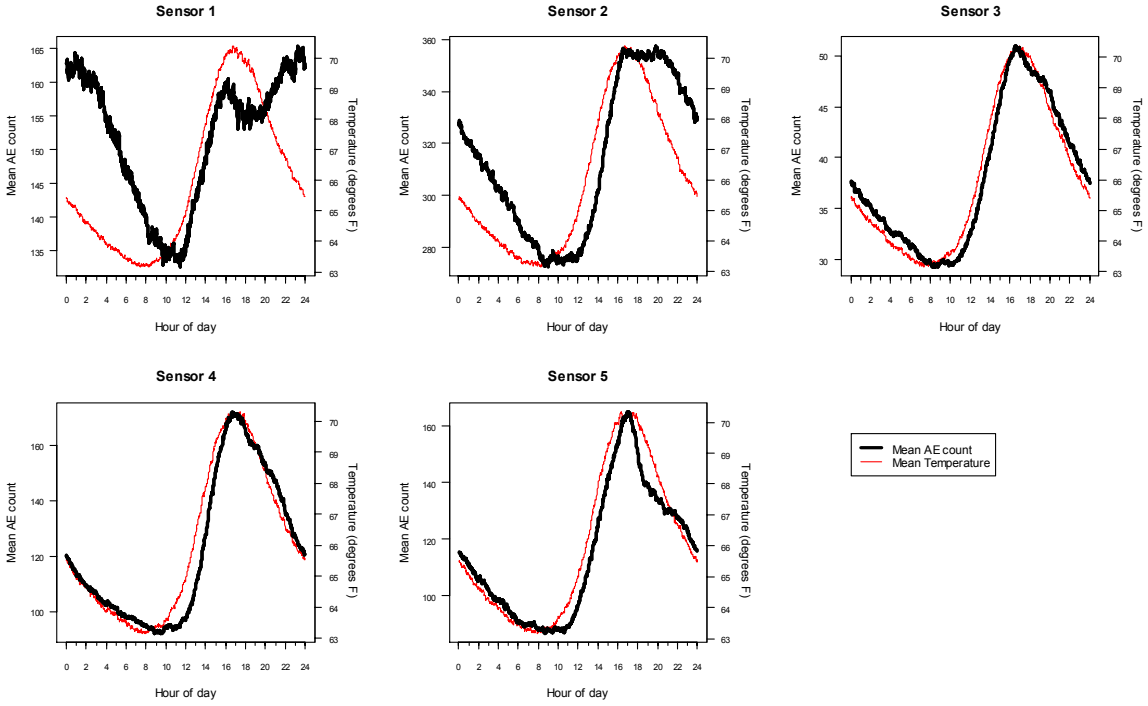

Figure 5. The mean hourly AE event count for each sensor (1-5) that occurred during the twenty-hour diurnal cycle. The heavy dark line is the mean AE event count. The red line is the temperature trace during the same 24 hour diurnal cycle measured in centigrade (°C). The average AE event for the untreated checks (logs with no live termites) from June 2008 to May 2009 is displayed at the bottom right side of the figure for comparison.

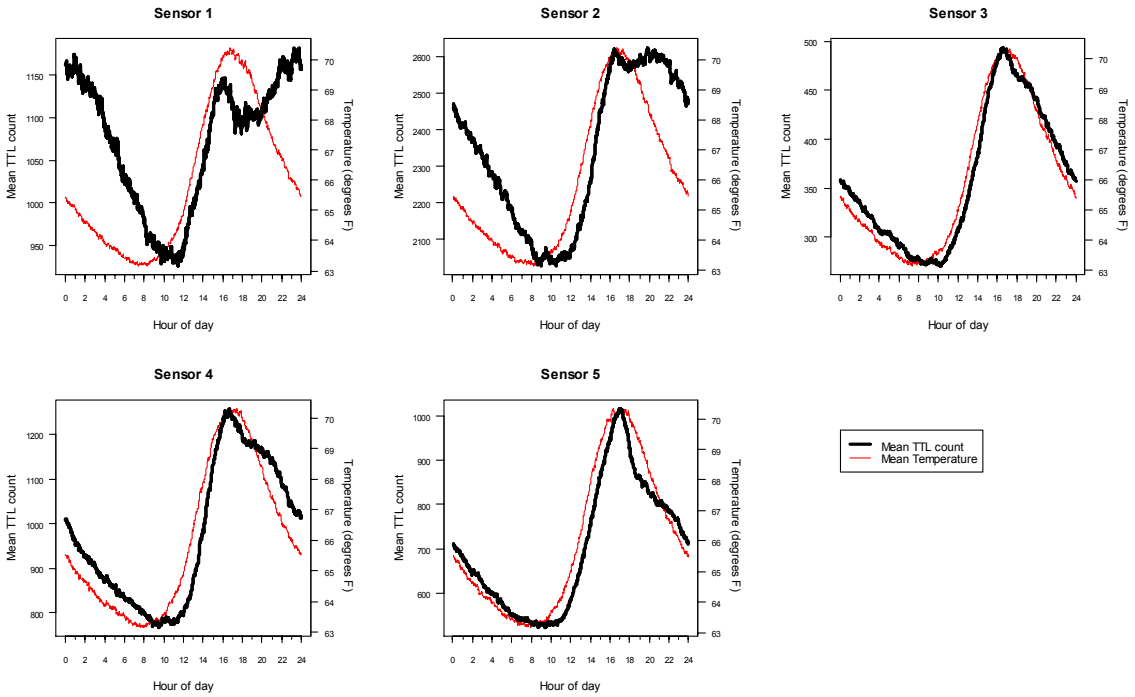

Figure 6. The mean hourly AE ring down count for each sensor (1-5) that occurred during the twenty-hour diurnal cycle. The heavy dark line is the mean AE ring down count. The red line is the temperature trace during the same 24 hour diurnal cycle measured in centigrade (^{o}C). The average AE ring down for the untreated checks (logs 6 and 7 with no live termites) from June 2008 to May 2009 is displayed at the bottom right side of the figure for comparison.

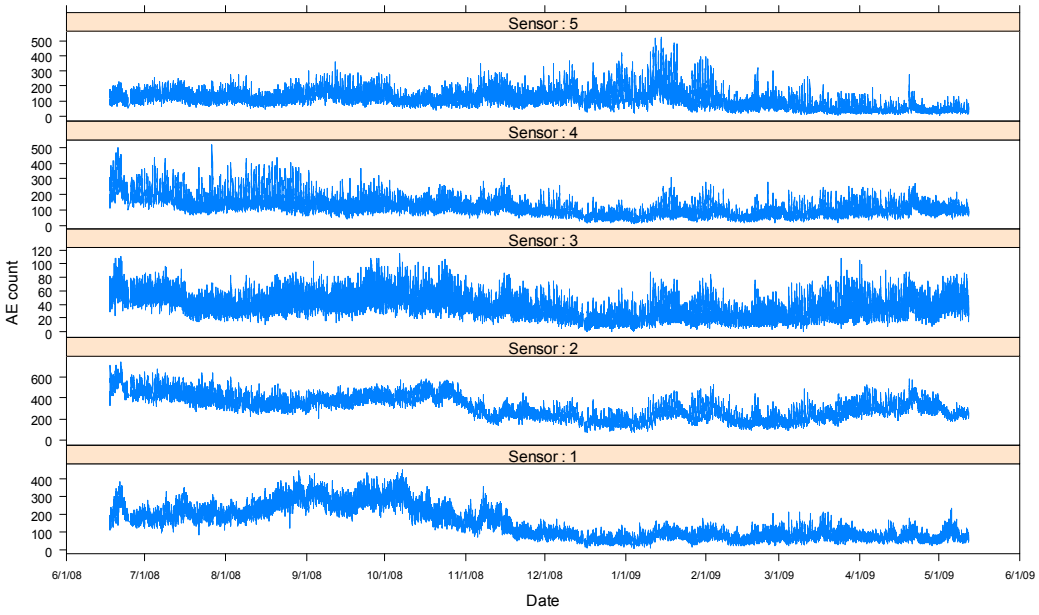

Figure 7. The seasonal mean hourly AE events for each sensor (1-5) that occurred during the 11-months from June 2008 to May 2009. The heavy dark line represents the mean AE ring down count. The light-hatched line represents the temperature trace during the same 11-month study measured in centigrade (^{o}C). The average AE ring down for the untreated checks (logs with no live termites) is displayed at the bottom of each plot for comparison.

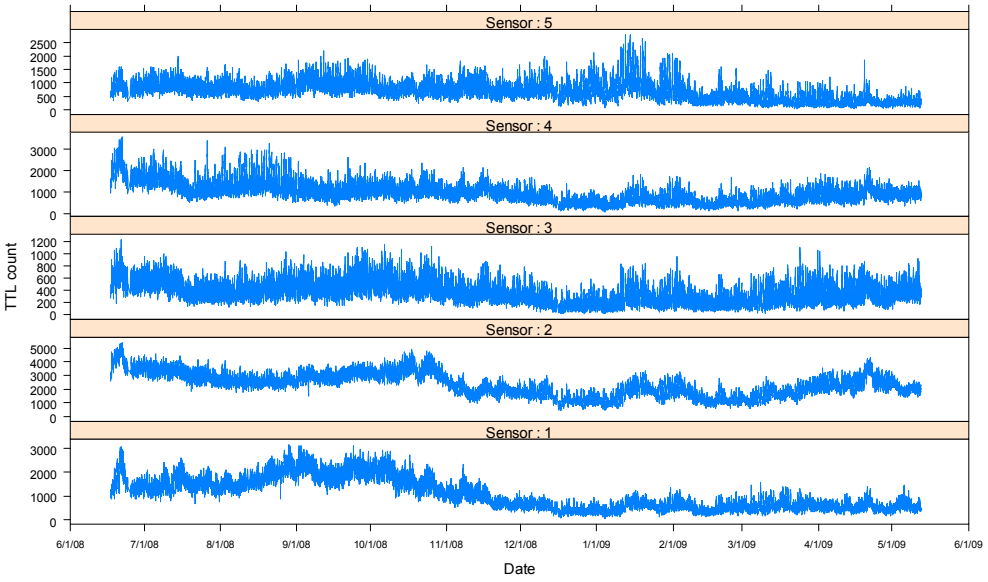

Figure 8. The seasonal mean hourly AE ring down count for each sensor (1-5) that occurred during the 11-months from June 2008 to May 2009. The heavy dark line is the mean AE ring down count. The light-hatched line represents the temperature trace during the same 11-month study measured in centigrade (°C). The average AE ring down for the untreated checks (logs with no live termites) is displayed at the bottom of each plot for comparison.

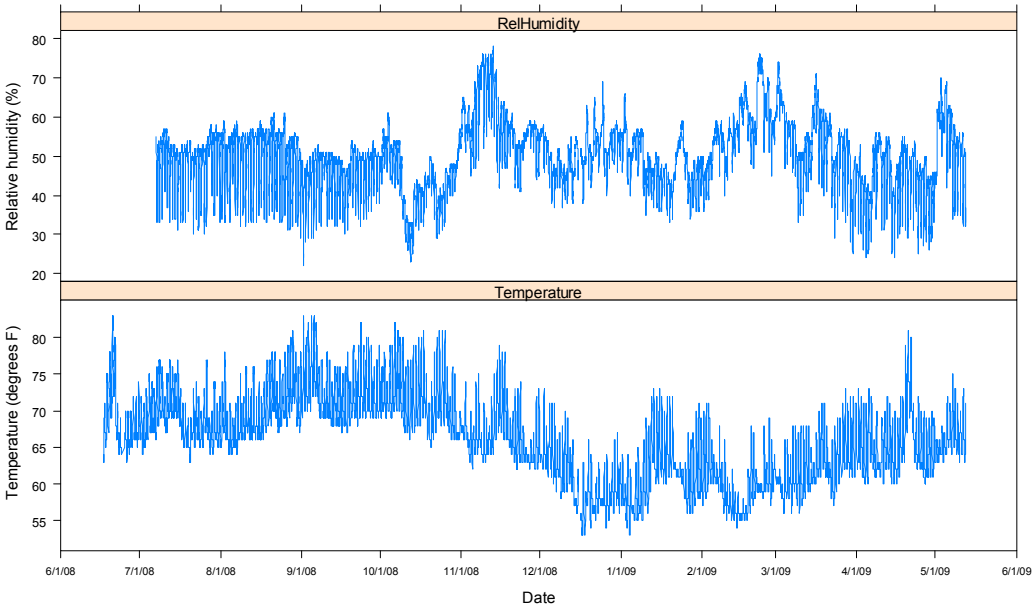

Figure 9. Seasonal temperature (°C) and relative humidity (%) traces for logs and building used to house the AE activity study at the University of California, Richmond Field Station from June 2008 to May 2009.

Table 1. Estimates of the coefficients (intercept, temperature, relative humidity, and the interaction term for temperature and relative humidity) for the sensors 1 to 5. Contained in the table results are estimate standard error, and p-value for AE events and ring down counts. Data collected over 11 months (June 2008 to May 2009) from loquat logs maintained at ambient conditions at the University of California Richmond Field Station.

		AE Events			**AE Ring down Counts**		
Sensor	**Variable**	**Estimate**	**Std. Err.**	***P*-value**	**Estimate**	**Std. Err.**	***P*-value**
1	Intercept	-6.6919	1.0600	0.000	-13.824	2.780	0.0000
	Temperature	0.1977	0.0157	0.000	0.509	0.041	0.0000
	Relative Humidity	-0.4106	0.0211	0.000	-1.192	0.055	0.0000
	Temperature*RelHumidity	0.0079	0.0003	0.000	0.022	0.001	0.0000
2	Intercept	25.0632	0.8250	0.000	66.077	2.232	0.0000
	Temperature	-0.0754	0.0122	0.000	-0.175	0.033	0.0000
	Relative Humidity	-0.6748	0.0164	0.000	-1.958	0.044	0.0000
	Temperature*RelHumidity	0.0094	0.0002	0.000	0.028	0.001	0.0000
3	Intercept	-5.3026	0.3989	0.000	-19.113	1.285	0.0000
	Temperature	0.1686	0.0059	0.000	0.561	0.019	0.0000
	Relative Humidity	-0.0419	0.0079	0.000	-0.140	0.026	0.0000
	Temperature*RelHumidity	0.0007	0.0001	0.000	0.002	0.000	0.0000
4	Intercept	-8.6581	0.7307	0.000	2.668	1.931	0.1670
	Temperature	0.2766	0.0108	0.000	0.366	0.029	0.0000
	Relative Humidity	-0.0840	0.0145	0.000	-0.775	0.038	0.0000
	Temperature*RelHumidity	0.0016	0.0002	0.000	0.013	0.001	0.0000
5	Intercept	-2.2482	1.2750	0.078	-19.368	2.760	0.0000
	Temperature	0.1863	0.0189	0.000	0.680	0.041	0.0000
	Relative Humidity	0.0023	0.0253	0.927	0.063	0.055	0.2512
	Temperature*RelHumidity	0.0000	0.0004	0.898	-0.001	0.001	0.2448

Table 2. The root mean square (MSE) and correlation coefficients for AE events and ring down counts for the sensors 1 to 5. Data collected over 11 months (June 2008 to May 2009) from loquat logs maintained at ambient conditions at the University of California Richmond Field Station.

Sensor	**AE Events**		**Ring Down Counts**	
	Root MSE	**Adj. R^2**	**Root MSE**	**Adj. R^2**
1	2.147	65.7%	5.631	67.4%
2	1.679	67.6%	4.542	71.9%
3	0.810	65.8%	2.609	67.3%
4	1.483	62.4%	3.903	64.9%
5	2.591	13.9%	5.608	28.8%