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Research

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Volatile Essential Oils Can Be Used to Improve the Efficacy of Heat Treatments Targeting the Western Drywood Termite: Evidence from a Laboratory Study

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Abstract

Use of heated air to create lethal temperatures within infested wood serves as a nonchemical treatment option against western drywood termites, *Incisitermes minor* (Hagen). When treating a whole or large portion of the structure, however, the presence of hard-to-heat areas (structural heat sinks) and potential risk of damaging heat-sensitive items are recognized as important challenges. To address these challenges, we tested if the incorporation of a volatile essential oil would increase the overall efficacy of heat treatments against the drywood termites. To choose an essential oil for use, we tested the volatile action of several candidate compounds against individual termites using a fumigant toxicity assay. As a proof-of-concept experiment, field-collected termites were housed in small wooden arenas and subsequently subjected to 2-h heat treatment at various air temperatures within a gas chromatography oven. A simulated heat sink and essential oil treatment was also included in the experimental design. Analyses of lethal temperatures ($LTemp_{50}$ and $LTemp_{99}$ values), probabilities of mortality, and survivorship data over time suggested that 1) the presence of a heat sink significantly increased the minimum air temperature needed for complete kill of the termites and 2) the volatile essential oil added at the site of a heat sink effectively counteracted the impact of the heat sink. The use of volatile essential oils makes it possible to effectively kill drywood termites even in areas which might not reach lethal temperatures (~50°C), potentially improving the overall efficacy of heat treatments while reducing the risk of heat damage.

Key words: drywood termite, methyl salicylate, heat, treatment

The western drywood termite, *Incisitermes minor* (Hagen) (Blattodea: Kalotermitidae), is one of the most common drywood termites in the United States. Its native range extends from Baja California and Sonora, north to Sacramento, and inland to the Sierras (Cabrera and Scheffrahn 2001). Western drywood termites are pests in their native range and have become established elsewhere in the United States and Canada; they are particularly problematic in California and Arizona (Grace et al. 1991, Cabrera and Scheffrahn 2001, Jones 2004). Western drywood termites have also become established in Japan (Indrayani et al. 2004, and references therein) and China (Xu et al. 2012). In California, the western drywood termite is an economically important pest, with a previously estimated annual economic impact of $250 million (Cabrera and Scheffrahn 2001), a figure that certainly has increased in the intervening years. Thus, control of this pest is of importance in California and other regions in which *I. minor* has become established.

If an infestation is confirmed, several control options are available for western drywood termites. One common method is a liquid or dust insecticide injection, introduced via holes drilled into the infested wood to intersect termite galleries. If the whole structure needs to be treated, fumigation can be used to introduce gaseous toxicants (i.e., fumigants) to eliminate all termite colonies within the structure. For an additional whole-structure treatment option, heating infested wood (heat treatment) can provide effective control of drywood termites if a lethal temperature is maintained inside the wood for a sufficient amount of time (Lewis and Forschler 2014, and references therein). However, if some sections of wood are shielded from the heat by structural features that act as insulators or heat sinks, it may be difficult to achieve the target temperatures to ensure elimination of the termite colonies (Lewis and Haverty 1996, Lewis and Forschler 2014). For example, Lewis and Haverty (1996) reported that some western drywood termites survived a heat treatment wherein the interior of the wood reached 50°C for 1 h and the air temperature of the test building reached >87°C. All of the surviving termites were found in the artificially infested pieces of wood that were placed against concrete foundations in the subarea of the test building. Furthermore, drywood termites can relocate within their galleries to avoid extreme temperatures (Cabrera and Rust 1996, Rust and Reierson 1998, Cabrera and Rust 2000). Together with the potential risk of damaging heat-sensitive items at high temperatures, the presence of these hard-to-heat areas has been considered as one of the major challenges in the use of heat treatment for drywood termite control (Gouge et al. 2001, Lewis et al. 2014).

Various botanical essential oils have been tested as natural insecticides (Isman 2000, 2006; Morgan 2004; Isman and Grieneisen 2014). In fact, several essential oils have been tested in laboratory studies for their insecticidal activity against various termite species. For example, Raina et al. (2007) reported that *D*-limonene killed Formosan subterranean termites, *Coptotermes formosanus* (Shiraki), via fumigant and contact actions. The same study found that exposure of Formosan subterranean termites to volatiles of *D*-limonene inhibited their feeding, though it was unclear whether this was due to repellency or some other effect (Raina et al. 2007). *D*-Limonene, the most abundant constituent of orange oil, is known to kill western drywood termites by contact and fumigant action (Lewis et al. 2009, Rust and Venturina 2009). Other compounds (e.g., eugenol from clove oil and citral from lemongrass oil) were found to cause mortality in Formosan subterranean termites by contact (Zhu et al. 2001), and several constituents of pine oil have been demonstrated to have contact toxicity against three species of *Reticulitermes* (Nagnan and Clement 1990). The mode of action of these compounds appears to be neurotoxic and may involve several target sites, including activation of tyramine and octopamine receptors and inhibition of acetylcholinesterase (Enan 2005, Rattan 2010, Gaire et al. 2019).

Essential oils hold a unique position among active ingredients (AIs) available for use in the urban/structural pest management market. They are typically characterized by some of the following characteristics: 1) they are based on ‘natural’ compounds, 2) some of them are on the ‘EPA 25(B) list’, being exempt from FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act) pesticide registration requirements, 3) they are typically more ‘volatile’ than other common synthetic AIs, and 4) they are typically repellent to many insect pests. Even though orange oil (*D*-limonene) has gotten attention from consumers and manufacturers for drywood termite control, a simple injection of small amounts of the essential oil into wood will most likely have limited residual efficacy due to its repellency and relatively quick loss of contact activity (Lewis and Rust 2009, Rust and Venturina 2009). Instead, we postulate that these essential oil compounds might be useful to improve current heat treatment methods by addressing the issues associated with structural heat sinks. For example, volatile essential oil compounds could be used to selectively treat accessible heat sinks to ensure the efficacy of heat treatments.

Using western drywood termites, the present study examined if the incorporation of insecticidal essential oils at the sites of heat sinks would improve the control efficacy of heat treatments. For a proof-of-concept experiment, several essential oils were compared, and a candidate was selected for use based on its chemical properties (e.g., volatility, flash point) and fumigant toxicity (see Methods and Results). Small wooden arenas were constructed to hold termites, simulating termite galleries in infested lumber. To understand the potential impact of structural heat sinks on heat treatment efficacy, some of the wooden arenas were placed on sand, simulating structural heat sinks. A subset of the arenas with heat sinks was treated with a small amount of essential oil. These arenas were heated in a gas chromatography oven at various temperatures for a fixed amount of time to determine the relationship between oven temperature and termite mortality in each treatment.

The present study had two goals. The first goal was to determine the extent to which structural heat sinks might impede heat treatments for the western drywood termite. It was hypothesized that the presence of a heat sink would significantly affect the efficacy of the heat treatment, necessitating a higher oven temperature to heat the wood for complete kill of the termites. The second goal was to investigate the use of insecticidal essential oils to mitigate the effect of heat sinks. It was hypothesized that the incorporation of an essential oil at the site of a heat sink would significantly lower the minimum oven temperature required to heat the wood for complete kill of the termites.

Methods and Materials

Insects

All termites were collected from wood acquired in Riverside, CA. A microwave termite detection device (T3i All Sensor, Termatrac, Ormeau, Australia) was used to screen wood for the presence of termites. Each piece of infested wood was cut into small sections, and the termites were collected using soft-touch forceps and a camel-hair brush. All termites were sorted by colony (collection location) and collection date into Petri dishes lined with filter paper discs (90 mm diameter; Millipore Sigma, Darmstadt, Germany). Balsa wood pieces were provided to serve both as food and as harborage. Each Petri dish was provisioned with a piece of water-soaked wood (0.1 × 0.5 × 1.5 cm) weekly as a source of moisture. Dead termites were removed from the dishes once per week and the termites were kept for at least 1 wk before the experiment to ensure that only healthy individuals were used.

Fumigant Toxicity Test

Bioassays were conducted to identify the botanical essential oil to be used for the subsequent experiments. Since the planned experimental design simulated scenarios where the essential oil treatment was made at the site of the heat sink, without directly contacting the termites in the wood, the vapor toxicity of the essential oil was of major importance. *D*-Limonene (95% purity, XT-2000, Santee, CA), methyl salicylate (MS; 99% purity, Millipore Sigma, St. Louis, MO), and eugenol (99% purity, Millipore Sigma) were used (Table 1). One termite pseudergate (immature and undifferentiated) was placed in a 20-ml scintillation vial (foamed polyethylene liner, DWK Life

Table 1. Essential oil compounds used for the fumigant toxicity assay

Compound	Vapor pressure	Flash point	Tested against *I. minor*?	Publications
D-Limonene	0.19 kPa at 20°C	48°C	Yes	Lewis et al. (2009), Rust and Venturina (2009)
Methyl salicylate	0.006 hPa at 20°C	96°C	No	—
Eugenol	0.001 kPa at 25°C	100°C	No	—

Sciences, Millville, NJ). A small section of balsa wood stick (0.3 × 0.6 × 3 cm length) was provided in the vial to serve as a substrate for the termite. A small ball of cotton (60–80 mg) was attached to the inner surface of the screw cap with hot glue (Ace Hardware, Oak Brook, IL) to create the finished test vial (Fig. 1). After adding 10 µl of the essential oil to the cotton, the vial cap was screwed tightly on the vial. Control vials were prepared following the same method, but the cotton ball did not receive any essential oil. All vials were kept at room temperature (≈26°C) for the duration of the test. Treatments and control were replicated 20 times.

The termites were checked once per hour for the first 7 h, then re-examined every day until 5 d of exposure. Because all the vials remained closed throughout the entire period, the observations were made through the glass. Three distinct conditions of the termites were recorded. Healthy individuals moved in response to light physical disturbance (i.e., gently rolling the vial) and they were able to cling to the wood. Moribund termites (i.e., unable to hold onto substrate but responded to gentle shaking of the vial) and dead termites (i.e., unable to hold onto substrate and no response to gentle shaking of the vial) were considered 'dead'.

Experimental Arena

To simulate termite galleries in infested structural lumber, experimental arenas were constructed from pieces of Douglas fir. The arenas (3.8 × 3.8 × 12.7 cm) were designed to house a group of western drywood termites during small-scale heat treatments. Each arena was consisted of two pieces of wood (1.9 cm height each). To hold the termites, a narrow channel (0.3 × 0.7 × 11 cm, with one open and one closed end) was routed along the centerline of one piece from each pair. A sheet of clear acrylic (0.2 × 3.8 × 12.7 cm, Plaskolite, Columbus, OH) was placed over the top of the channel-bearing bottom piece of the arena to facilitate observation. The acrylic sheets were rinsed with 2% Liquinox detergent (Alconox, White Plains, NY) and deionized water before placement in the arena to standardize the environment within. The top piece was placed on the bottom piece, held together with two rubber bands and with the acrylic sheet pressed between them, to form a complete arena (Fig. 2).

Fig. 1. One vial ready for use in the fumigant toxicity test, including balsa wood, one termite, and cotton inside the cap.

In total, 10 pseudergates were added into each arena through the open end of the channel before the heat treatment trials. Of these 10, at least one individual was an early-instar pseudergate (2–3 mm length), and at least one was a late-instar pseudergate with wing buds (7–9 mm length). The rest were intermediate-instar individuals without wing buds (4–6 mm length). Once the termites were placed in the arena, a small piece of cotton (≈33 mg) was used to plug the open end of the channel. This cotton piece also served as the substrate for MS application.

Experimental Design

Three different treatment conditions were tested: 1) heat only, 2) heat + heat sink, and 3) heat + heat sink + MS. During a heat treatment in a structure, a colony located within a freestanding beam of structural lumber, such as a wall stud, would be exposed to heat without much shielding ('heat only'). To simulate this condition, arenas were vertically placed atop a disc of 0.6 cm wire mesh (7 cm diameter) seated on a glass cylinder (6.5 cm diameter × 8 cm height, Fig. 3A). The open end of the channel with cotton plug was oriented downward, and tape was used to secure the arena on the mesh. During a heat treatment, infested wood adjoining a structural heat sink might experience some degree of shielding from the heat treatment ('heat +

Fig. 2. One assembled arena (left) and disassembled component pieces.

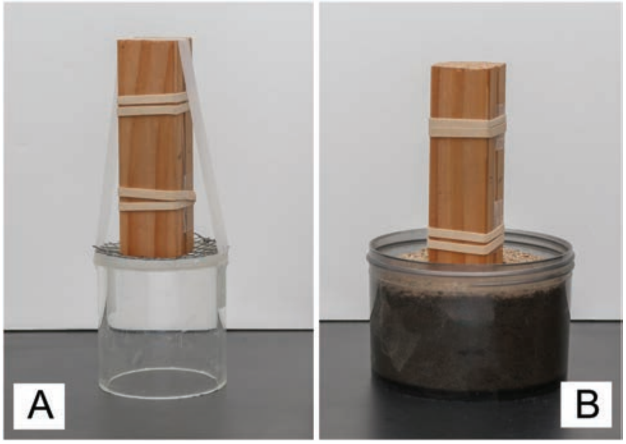

Fig. 3. Arena setups for each trial type. (A) An arena resting on wire mesh on top of a glass cylinder for heat only trials. (B) An arena seated in 600 g of sand for all trials utilizing a heat sink.

heat sink'). To simulate this condition, arenas were vertically placed on the surface of sand (600 g, Quikrete, Atlanta, GA) contained in a plastic jar (11.2 cm diameter × 7.2 cm height; Fig. 3B). The end of the arena with the cotton plug was embedded in the sand, at a depth of ~2 cm. To determine whether the incorporation of MS treatment at the site of heat sinks would improve heat treatment efficacy, 20 µl of MS was added to the cotton plug before placement in sand using a 10- to 100-µl micropipette (Eppendorf, Hauppauge, NY).

Heat Treatments

The oven of a 5890A gas chromatograph (GC; Hewlett-Packard, Palo Alto, CA) was used to provide consistent heat treatment (Scheffrahn et al. 1997). The interior of the oven measured 35 × 28 × 23 cm (0.0225 m^3). Because there was a minor discrepancy between the set temperature on the control panel and the actual temperature in the oven, preliminary tests were conducted using thermocouples (1.27 × 1.90 mm copper-constantan; Omega Engineering, Norwalk, CT) with the goal of determining the precise temperature within the oven at various temperature settings. Two thermocouples were placed inside, with each positioned 5.5 cm from the nearest wall (left or right), 5.5 cm from the front of the oven, and 10 cm above the floor of the oven. With the thermocouples inside, the oven was left on for 30 min after it reached the set temperature. Measurements were taken from each thermocouple at 10, 20, and 30 min. The mean value from these measurements was used as the representative temperature for the treatment.

Arenas containing 10 pseudergates were placed in the GC oven, and the oven temperature was increased immediately to the target temperature. Preliminary testing with thermocouples indicated that under 'heat + heat sink' conditions, the inside of an arena would reach a target temperature of 50°C after approximately 1 h. Accordingly, the heat treatment trials were conducted for 2 h, starting at the time when the oven reached the set target temperature, which took about 5–10 s. Starting around 47°C, higher or lower temperatures were tested by increasing or decreasing 1°C at a time. The goal was to include a range of several different temperatures resulting in low mortality of termites at lower temperatures and complete mortality at the higher temperatures. Because the termites were tested under a variety of conditions (i.e., with or without a heat sink, with or without MS), different ranges of temperatures were tested for each treatment. Control trials were conducted with and without MS added, with the oven set to 25°C to simulate room temperature (thermocouple measurements found the exact temperature to be 28.3°C). Each treatment and control were replicated eight times.

Two arenas from a single treatment were treated at a time in the GC oven. The arenas were placed in the same positions as the thermocouples used to determine oven temperatures at each setting (5.5 cm from the nearest wall and 5.5 cm from the front of the oven). Because different areas in the oven might vary slightly in temperature and air movement, orientation and location of two arenas were rotated between four different configurations. The four configurations stemmed from, first, whether a given arena was placed on the left side or the right side of the oven and, second, whether the channel-bearing sides of the arenas faced the front (door) or the back of the oven.

After each heat treatment, the oven was turned off and the arenas were removed. Between treatments, the oven door was left open for ~30 min to allow aeration. The sand used to simulate heat sinks was discarded and replaced after exposure to MS to avoid contamination. Including the controls, 20 combinations of different temperatures and treatments were tested.

Data Collection

After the heat treatment, termites from the arenas were immediately transferred to a plastic Petri dish (60 mm diameter × 15 mm height; Corning Inc., Corning, NY) for observation. Each Petri dish was provisioned with a small piece of paper towel (~6 cm^2) to serve as a substrate and food source. The number of dead and moribund termites in each arena was counted immediately after the termites were transferred to the Petri dish, and daily for the next 14 d. Mortality was scored as in the fumigant toxicity test. Separate pairs of forceps were used for different treatment groups to prevent cross-contamination.

Statistical Analysis

All statistical analyses were conducted using R version 3.4.1 (R Core Team 2013). Survivorship data from the fumigant bioassays were analyzed using a Kaplan–Meier survival analysis (Kassambara and Kosinski 2018). This analysis compared the distribution of survival times of termites subjected to each of three essential oils using the survivorship function $S(t)$, the probability of an individual termite surviving past a given time point (t). A log-rank test was used to determine whether there was an overall difference among the three essential oils and the untreated control (Mantel 1966, Peto and Peto 1972). Once the null hypothesis (i.e., there is no difference among the survival curves) was rejected, multiple comparisons of the survival curves were conducted using a log-rank test with BH-adjusted P values (Kassambara and Kosinski 2018). Based on this preliminary test, the most effective essential oil was chosen for the subsequent experiments involving heat treatment.

For the heat treatment, the overall cumulative mortality data were graphed for days 1–14 post-treatment to illustrate trends in mortality over time (Wickham 2016). The pooled data from eight replications (% mortality of 80 termites) were used for each combination of treatment and temperature. Based on the level of background mortality and its trend over time in the control (i.e., exposed to ambient temperature) by 14 d, the 7-d data were used for subsequent analyses, which required the choice of a single time point (see Results).

Generalized linear models (GLMs) were used to compare the three treatments ('heat only', 'heat + heat sink', and 'heat + heat sink + MS') across different temperature settings. Initially, logit analyses were conducted with temperature as the explanatory variable and mortality (yes/no) as the outcome (Hlina 2019). The temperatures required to produce 50 and 99% mortality (LTemp$_{50}$ and LTemp$_{99}$) were calculated along with associated 95% confidence intervals. Mortality ratio tests were used to compare different treatments in their LTemp$_{50}$s and LTemp$_{90}$s (Wheeler et al. 2009). These analyses allowed for comparison of the different treatments in terms of temperatures that would be required to achieve a certain level of mortality.

A logistic regression was used to illustrate the probability of mortality for each treatment (Fox and Weisberg 2018). Logistic regression was chosen over analysis of variance (ANOVA) because the data were not expected to follow a normal distribution, as assumed by ANOVA. It is possible to use an 'arcsine square root transformation' prior to conducting ANOVA to address the issue of normality. However, recent evidence indicates that for the statistical power of logistic regression tends to be higher than arcsine-transformed linear models for binomial data (Warton and Hui 2011). Three explanatory variables were included in the model: temperature, treatment (heat only, heat + heat sink, or heat + heat sink + MS), and colony (by location). The outcome was mortality (yes/no). All termites collected from a particular location (e.g., a large pile of wooden blocks) were considered to comprise one colony (A through D). To determine which model provided the best fit for the data, an automated model selection was performed (Barton 2018). This analysis compares models generated using combinations of fixed-term effects from the most inclusive model (i.e., the global model) and ranks them according to their Akaike information criterion. A global model was used including temperature, treatment, and colony (by location) as explanatory variables, alongside a term to represent the interaction between temperature and treatment.

The resulting 'best' model was used to determine whether the presence of a structural heat sink and/or the MS treatment significantly affected the final mortality of termites under varying temperature conditions. First, the probability of mortality was compared between 'heat only' and 'heat + heat sink' treatments to determine whether the presence of a heat sink resulted in significantly lower probability of mortality. Second, the probability of mortality was compared between 'heat + heat sink' and 'heat + heat sink + MS' treatments to determine whether the addition of MS significantly increased the probability of mortality despite the heat sink. To understand how the predicted probabilities of mortality change as the independent variable (i.e., treatment) changes, effects plots were generated (Fox and Weisberg 2019). The effects plots provide the predicted values of the outcome (i.e., probability of mortality) for certain given values for the explanatory variable (i.e., treatment).

Survivorship data for the heat treatment trials conducted at 45.7°C were analyzed using a Kaplan–Meier survival analysis (Kassambara and Kosinski 2018). This temperature was chosen because all three treatments were tested at 45.7°C, and moderate mortality was observed for 'heat only' (see Results). Log-rank tests were used for an overall comparison of the three treatments, as well as for subsequent multiple comparisons among the survival curves, using the same methods as in the fumigant assay analysis (see above).

Results

Fumigant Toxicity Test

Results from the Kaplan–Meier survival analysis are shown in Fig. 4. After 1 h, only *D*-limonene showed a slight drop in survivorship. However, over the next several hours, the survivorship in MS steeply declined, with 0 survivorship after 7 h. By this time, the survivorship for *D*-limonene dropped to ~0.8, whereas there was no change in survivorship for eugenol or the control. A global log-rank test indicated that there was a significant difference among the survival curves ($\chi^2 = 75.3$, df = 3, $P < 0.001$). A pairwise comparison of the survival curves indicated that MS and the other treatments were significantly different (log-rank test: $P < 0.001$). Furthermore, survival curves were significantly different between *D*-limonene and eugenol, as well as *D*-limonene and control (log-rank test: $P = 0.045$ for both). Survival curves were identical between eugenol and control ($P = 1.00$), with survivorship probability 1 by 7 h. Additional follow-up observations on days 1, 4, and 5 of exposure revealed that after 4 d of exposure the fumigant action of eugenol was sufficient to kill all termites, whereas *D*-limonene never fell below ~0.7 survivorship even after 5 d. Based on the results, MS was chosen for the subsequent experiments.

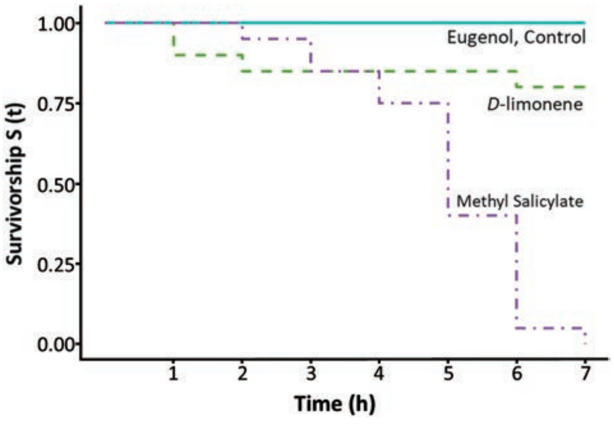

Fig. 4. Survival plots from fumigant toxicity tests. S(t) represents the estimated probability of an individual termite surviving past a given hour (1–7). Survival curves for eugenol and control (untreated) completely overlap.

Heat Treatments

Overall cumulative mortality data for the heat treatment trials are summarized in Fig. 5. In 'heat only' (Fig. 5A) and 'heat + heat sink' (Fig. 5B), many of the 1-d survivors were affected by the treatment, and mortality continued to increase in the subsequent observations. In 'heat + heat sink + MS' (Fig. 5C), although a substantial portion of the total mortality was accounted for by the 1-d observations, some of the 1-d survivors were also apparently affected by the treatment, and some additional mortality accrued over time. Cumulative mortality by 7 d accounted for 92% of the total mortality (14 d post-treatment mortality). In addition, in the no-heat control trials, mortality rose from 1% by 7 d to 9% by 14 d. Background mortality close to 10% could substantially affect the data analyses and interpretation. For these reasons, the cumulative mortality data up to day 7 post-treatment were used for subsequent statistical analyses.

A range of six temperatures was tested for each treatment with low mortality at one end of the range, and complete mortality at the other end. The lowest range of temperatures was used for 'heat + heat sink + MS' (range: 42.2–47.4°C), and the highest range was used for 'heat + heat sink' (range: 45.7–50.5°C). For 'heat only' (range: 43.3–47.4°C), low mortality (9%) was observed at 43.3°C, and complete mortality was achieved at 47.4°C. For 'heat + heat sink', low mortality (5%) was observed even at the relatively high temperature of 45.7°C, and complete mortality occurred at 49.6°C. 'Heat + heat sink + MS' had the lowest temperature range, with 55% mortality at 42.2°C and complete mortality at 45.7°C. Finally, the no-heat controls (28.3°C) had 10% or 1% mortality with or without MS, respectively.

Generalized Linear Models

Based on the cumulative mortality data up to day 7 post-treatment, LTemp$_{50}$s and LTemp$_{99}$s for three different treatments were calculated (Table 2). When exposed only to heat, termites had an LTemp$_{50}$ of 45.6°C and an LTemp$_{99}$ of 49.0°C. 'Heat + heat sink' had higher LTemp$_{50}$ and LTemp$_{99}$ values at 47.5 and 49.6°C, respectively. However, the lowest values were observed in 'heat + heat sink + MS', with an LTemp$_{50}$ of 42.6°C and an LTemp$_{99}$ of 47.5°C. Ratio tests returned evidence for significant differences between the LTemp values for 'heat only' and 'heat + heat sink' (LTemp$_{50}$: $Z = 28.5$, $P < 0.001$, LTemp$_{99}$: $Z = 8.0$, $P < 0.001$), as well as between the LTemp values for 'heat + heat sink' and 'heat + heat sink + MS' (LTemp$_{50}$: $Z = 44.1$, $P = 0$, LTemp$_{99}$: $Z = 17.4$, $P < 0.001$).

Effects plots generated from logistic regression models were used to compare the treatments in their probabilities of mortality. The automated model selection was used to include temperature, treatment, and colony (by location) as explanatory variables, alongside a term to represent the interaction between temperature and treatment.

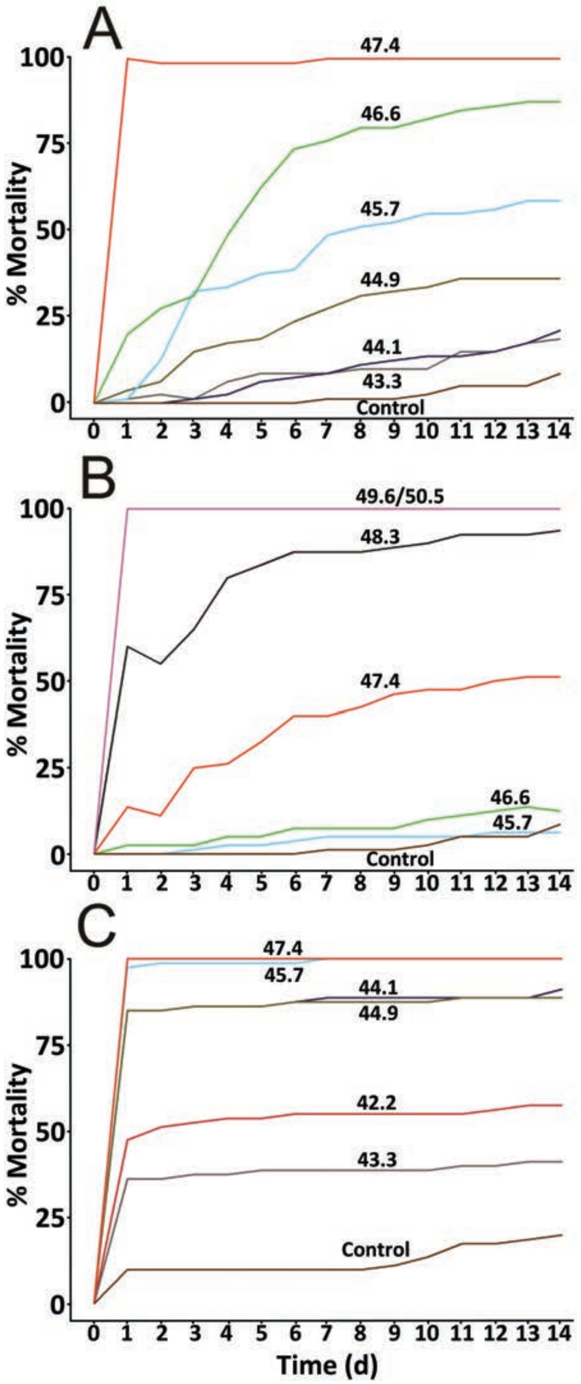

Fig. 5. Cumulative overall mortality for all temperatures (labeled next to lines in °C) within treatments, including the no-heat controls. (A) Mortality from heat only trials. (B) Mortality from heat + heat sink trials. (C) Mortality from heat + heat sink + MS trials.

Based on the effects plots and the output of the corresponding GLM, 'heat only' had a significantly higher expected probability of mortality than 'heat + heat sink' ($Z = -3.62$, $P < 0.001$). Similarly, 'heat + heat sink + MS' had a significantly higher expected probability of mortality than 'heat + heat sink' ($Z = -6.30$, $P < 0.001$).

Table 2. LTemp_{50} and LTemp_{99} values for each treatment with 95% confidence intervals

Treatment	LTemp_{50} (°C)[a]	LTemp_{99} (°C)
Heat only	45.6 (45.4, 45.7)	49.0 (48.4, 49.6)
Heat + heat sink	47.5 (47.4, 47.7)	49.6 (49.1, 50.0)
Heat + heat sink + MS	42.6 (42.3, 43.0)	47.5 (46.5, 48.4)

[a] The LTemp values represent the temperatures at which a given percentage of termites exposed to a given treatment are expected to experience mortality.

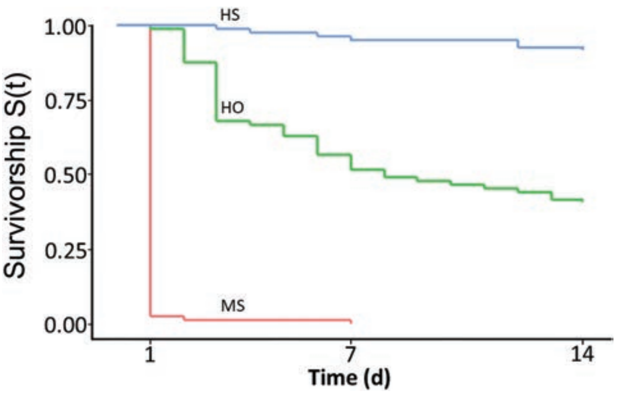

Fig. 6. Survivorship curves for each treatment type at 45.7°C. Each interval represents the probability of an individual termite surviving until a given day (1–7) during the observation period. Treatment abbreviations: HO = heat only; HS = heat + heat sink; MS = heat + heat sink + MS.

Survival Analyses

Results for the Kaplan–Meier survival analysis for the heat treatments at 45.7°C are shown in Fig. 6. Three different trends were visible in the survivorship curves. Termites in 'heat only' initially had high survivorship, which gradually decreased to ~0.5 over the observation period. In 'heat + heat sink', survivorship remained high (>0.9). 'Heat + heat sink + MS' had the lowest survivorship, which dropped to 0 by day 7 post-treatment. A global log-rank test indicated that there is a significant difference among the survival curves ($\chi^2 = 260$, df = 2, $P < 0.001$). A pairwise comparison of the survivorship curves revealed evidence for significant differences between each possible pairing (log-rank test: $P < 0.001$), providing further evidence that treatment with the essential oil constituent significantly affects mortality even in the presence of a heat sink.

Discussion

Based on the fumigant toxicity test, MS provided complete mortality (death + moribundity) of drywood termites in the shortest amount of time among the compounds tested. The vapors of eugenol and *D*-limonene eventually resulted in either complete or almost complete mortality after several days of exposure, and the rate at which they killed termites was significantly slower than that of MS. Although eugenol eventually killed 100% of exposed termites and could work as a fumigant, MS was selected for the study because it provided the fastest kill.

Daily mortality data from the heat treatment experiments indicated that mortality observed at 1 d post-treatment was not always indicative of final mortality, especially in 'heat only' and 'heat + heat sink' trials (see Fig. 5A and B). After 1 d post-treatment, moribundity and mortality continued to accrue, and occasionally also decrease (i.e., termites sometimes recovered after initial observation). This may have been caused by residual effects of exposure to heat, especially in trials in which the termites approached temperatures sufficient to cause immediate death. For these reasons, mortality data up to day 7 post-treatment were chosen for analyses.

Target temperature and heating duration are important parameters in heat treatment protocols for drywood termites. Generally, 6 h of heating is considered sufficient to bring all the wood in a structure to a target temperature and maintain that temperature for the duration necessary to control drywood termites (Rust and Reierson 1998). For most heat treatments, a target temperature of ~50°C (internal wood temperature) is used. Even though higher target temperatures can be used (e.g., 54.5°C) to account for 'worst case' situations where suspected infestations are inaccessible for temperature monitoring, prior research has indicated that relatively brief exposure (10–15 min) to temperatures around 50°C is sufficient to kill several economically important species of drywood termites (Scheffrahn et al. 1997). If the target temperature could be lowered for a heat treatment, it would be possible to reduce energy and time costs associated with the heating process (Scheffrahn et al. 1997). The difference between LTemp_{50} values for 'heat + heat sink' and 'heat + heat sink + MS' was ~5°C. The difference between LTemp_{99} values was smaller (~2°C), but still statistically significant. In addition to cost benefits associated with reduced heating time, the LTemp differences between 'heat + heat sink' and 'heat + heat sink + MS' might also imply a significant benefit by reducing the temperature needed for control. For example, the ability to use a lower temperature would lower the likelihood of damaging heat-sensitive objects or structural features during the treatment.

Based on the effects plot analysis, 'heat + heat sink' treatment had significantly lower mortality probability than 'heat only' treatment. This result supports the hypothesis that structural heat sinks shield termites from heat treatments. The presence of heat sinks in the structure might make heat treatments more difficult, necessitating either higher temperatures or longer heating duration to achieve lethal conditions in the 'hard-to-heat' areas. Similarly, the effects plot analysis indicated that 'heat + heat sink + MS' treatment had significantly higher mortality probability than did 'heat + heat sink' treatment. This supports the hypothesis that the addition of MS at the site of a heat sink can effectively counteract the insulative nature of the structural heat sink.

The Kaplan–Meier survivorship analyses provided a means of comparing the mortality trends of different treatments across 7 d of observation, rather than at a single time point (Fig. 6). At the temperature used for this analysis (45.7°C), the three treatments showed highly distinct survivorship trends. The divergence between 'heat only' and 'heat + heat sink' after early observations, as well as the rapid drop in survivorship for 'heat + heat sink + MS', clearly demonstrated the differences in mortality among them. In particular, a rapid drop in survivorship immediately after the heat treatment was characteristic for the 'heat + heat sink + MS' treatment. In both the 'heat only' and 'heat + heat sink' treatments, much of the mortality accumulated gradually over the entire observation period (also see Fig. 5A and B). In contrast, most (>90%) of the final mortality was already accounted for by 1 d post-treatment in the 'heat + heat sink + MS' treatment (also see Fig. 5C). This difference indicates that the presence of MS in the termite gallery provided faster mortality of termites when used in conjunction with heat treatment.

What explains the rapid drop in survivorship of termites when they are exposed to MS and sublethal temperature conditions simultaneously? There are at least three potential explanations. First, it is possible that high temperature drives termites away from the hotter end of the arena down to the cooler end near the sand (i.e., heat sink; Cabrera and Rust 2000), where close proximity to the essential oil quickly kills the termites. Second, the toxicity of MS could synergize with heat. The activity of certain insecticides has been found to correlate positively with increasing temperature (Khan and Akram 2014); given the volatile nature of essential oil compounds and increased termite respiration at high temperature, the insecticidal action of essential oils might also increase with temperature. Last, if MS has a repellent effect, its presence at the heat sink area would prevent the termites from seeking refuge in the cooler part of the arena. Several essential oil constituents have been found to exhibit repellent activity against *C. formosanus*, including nootkatone from vetiver oil (Zhu et al. 2001), and ilicic acid methyl ester and costic acid from white cypress pine (Watanabe et al. 2005). It is possible that MS has a similar repellent effect on western drywood termites. This repellent effect could drive the termites back to the hotter end of the arena, where the combination of volatile MS and high temperature (which are both suboptimal in providing quick mortality when used separately) would quickly kill the termites. Future research on termite behavior (i.e., location and various parameters in locomotion) or toxicity of volatile essential oils at varying temperatures would be necessary to answer this question.

The results of this study might have significant implications for the use of essential oils in conjunction with heat to control drywood termites. However, the results should be interpreted with caution due to the controlled laboratory conditions used in the present study. First, the essential oil compound was introduced into the channel via pieces of cotton. In a real-world treatment, the essential oils would be added to holes drilled in the wood at regular intervals, meaning the galleries might not be reached directly. We understand that MS and other essential oil compounds will kill termites by direct contact (i.e., when the oils are applied directly on the insects or when the termite gallery was inundated with injected essential oil). We designed the current experiment to be as conservative as possible to determine whether a small amount of MS within the termite gallery (i.e., part of the gallery wall has received MS from a nearby injection site) could be effective in improving heat treatment efficacy. Based on the properties of these essential oil compounds and our preliminary observations, it would be reasonable to assume that the essential oil injected into the wood would seep through the wood and travel some distance from the initial injection sites. By adding the MS to the cotton balls, the amount of insecticide directly traveling through the channel was minimized. Second, the wooden arenas were likely to have been heated fairly evenly compared with wood in a heat treatment of a structure. When heating a home, many factors could produce variation in heating rates such as wood thickness, distance from heaters, or building style (i.e., a stucco or brick exterior shielding wood). Last, the wooden arenas were removed from heat and brought to ambient temperature (24–26°C) immediately following the 2-h treatment period. In an actual structural heat treatment for drywood termites, the infested wood remains within the heated structure even after the heating process ends. Thus, it is likely that the wood in the structural heat treatment may cool down more slowly compared with what was simulated in the current experiment. This discrepancy might influence the interpretation of the current laboratory results, with lower mortality than what would be observed in structural heat treatments at similar target temperatures. However, this discrepancy should result in a more conservative estimate of termite mortality in various experimental conditions. It is also possible that, in a real structure, the impact of heat sinks on drywood termite control might be lessened due to residual heat after heating is concluded.

The present study had two hypotheses: first, the presence of a structural heat sink might result in significantly increased temperature needed for complete mortality and second, the addition of MS at the site of the heat sink would result in significantly reduced temperature needed for complete control. Although both hypotheses were supported, the results need to be considered in the context of real-world heat treatments. The controlled laboratory environments used in the current trials could not fully simulate the conditions that would be experienced by drywood termites in a real structure. For example, the use of a GC oven and small wooden arenas in the present study might have provided a relatively quick and evenly distributed heat treatment. A rapid and spatially even heating process is less likely for real structural heat treatments, in which a home is treated with several propane heaters and fans. In addition, wood members in a treated structure may not heat at the same rate as the scaled-down wooden arenas used in the experiment. The logistics of applying essential oils at all required points throughout a structure (i.e., all accessible heat sinks) also need to be considered. Finally, the insulating properties of typical structural heat sinks (i.e., a concrete foundation, a porcelain bathroom fixture, or insulation) are likely to be different from those of the sand used in the present study. To demonstrate the benefit and practicality of the combined use of botanical essential oils and heat treatment for drywood termites, a more realistic demonstration study needs to be conducted using a larger structure and actual heating protocols that are currently used in typical heat treatments.

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References Cited

Barton, K. 2018. MuMIn: multi-model inference. R package version 1.42.1. (https://CRAN.R-project.org/package=MuMIn).

Cabrera, B. J., and M. K. Rust. 1996. Behavioral responses to light and thermal gradients by the western drywood termite (Isoptera: Kalotermitidae). Environ. Entomol. 25: 436–445.

Cabrera, B. J., and M. K. Rust. 2000. Behavioral responses to heat in artificial galleries by the western drywood termite (Isoptera: Kalotermitidae). J. Agric. Urban Entomol. 17: 157–171.

Cabrera, B. J., and R. H. Scheffrahn. 2001. Western drywood termite, *Incisitermes minor* (Hagen) (Insecta: Isoptera: Kalotermitidae). UF/IFAS Extension EENY 248: 1–7.

Enan, E. E. 2005. Molecular and pharmacological analysis of an octopamine receptor from American cockroach and fruit fly in response to plant essential oils. Arch. Insect Biochem. Physiol. 59: 161–171.

Fox, J., and S. Weisberg. 2018. Visualizing fit and lack of fit in complex regression models with predictor effect plots and partial residuals. J. Stat. Softw. 87: 1–27.

Fox, J., and S. Weisberg. 2019. An R companion to applied regression, 3rd ed. Sage, Thousand Oaks, CA. (http://tinyurl.com/carbook).

Gaire, S., M. E. Scharf, and A. D. Gondhalekar. 2019. Toxicity and neurophysiological impacts of plant essential oil components on bed bugs (Cimicidae: Hemiptera). Sci. Rep. 9: 1–12.

Gouge, D. H., K. A. Smith, C. Olson, and P. Baker. 2001. Drywood termites. Cooperative Extension, College of Agriculture & Life Sciences, The University of Arizona. (http://ag.arizona.edu/pubs/insects/az1232/).

Grace, J. K., G. M. Cutten, R. H. Scheffrahn, and D. K. McE. Kevan. 1991. First infestation by *Incisitermes minor* of a Canadian building (Isoptera: Kalotermitidae). Sociobiology 18: 299–304.

Hlina, B. L. 2019. Ecotox: analysis of ecotoxicology. R package version 1.4.0. (https://CRAN.R-project.org/package=ecotox).

Indrayani, Y., T. Yoshimura, Y. Fujii, Y. Yanase, Y. Okahisa, and Y. Imamura. 2004. Survey on the infestation of houses by *Incisitermes minor* (Hagen) in Kansai and Hokuriku areas. Jpn. J. Environ. Entomol. Zool. 15: 261–268.

Isman, M. B. 2000. Plant essential oils for pest and disease management. Crop Prot. 19: 603–608.

Isman, M. B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomol. 51: 45–66.

Isman, M. B., and M. L. Grieneisen. 2014. Botanical insecticide research: many publications, limited useful data. Trends Plant Sci. 19: 140–145.

Jones, S. C. 2004. New inland records of *Incisitermes minor* (Isoptera: Kalotermitidae) along the Colorado River. Sociobiology 43: 565–572.

Kassambara, A., and M. Kosinski. 2018. Survminer: drawing survival curves using 'ggplot2'. R package version 0.4.3. (https://CRAN.R-project.org/package=survminer).

Khan, H. A. A., and W. Akram. 2014. The effect of temperature on the toxicity of insecticides against *Musca domestica* L.: implications for the effective management of diarrhea. PLoS One 9: 1–6.

Lewis, V. R., and B. Forschler. 2014. Management of drywood termites: past practices, present situation and future prospects, pp. 130–153. *In* P. Dhang (ed.), Urban insect pests: sustainable management strategies. CABI, Boston, MA.

Lewis, V. R., and M. I. Haverty. 1996. Evaluation of six techniques for control of the western drywood termite (Isoptera: Kalotermitidae) in structures. J. Econ. Entomol. 89: 922–934.

Lewis, V. R., and M. Rust. 2009. Drywood termite control: preliminary laboratory evaluation of chemical local treatments for drywood termites. Voice PCOC Spring 2009: 14–17.

Lewis, V. R., S. Moore, R. Tabuchi, and G. Getty. 2009. Field evaluations of localized treatments for control of drywood termite infestations in California. *In* Progress report prepared for the California Structural Pest Control Board, Sacramento, CA, p. 29. (https://www.pestboard.ca.gov/howdoi/research/2009_field_rpt.pdf).

Lewis, V. R., A. M. Sutherland, and M. I. Haverty. 2014. Pest notes: drywood termites, pp. 1–5. University of California Division of Agriculture and Natural Resources Publication 7440, Oakland, CA.

Mantel, N. 1966. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother. Rep. 50: 163–170.

Morgan, E. D. 2004. The place of neem among modern natural pesticides, pp. 21–32. *In* O. Koul and S. Wahab (eds.), Neem: today and in the new millennium. Springer, Berlin, DE.

Nagnan, P., and J. L. Clement. 1990. Terpenes from the maritime pine *Pinus pinaster*: toxins for subterranean termites of the genus *Reticulitermes* (Isoptera: Rhinotermitidae). Biochem. Syst. Ecol. 18: 13–16.

Peto, R., and J. Peto. 1972. Asymptotically efficient rank invariant test procedures (with discussion). J. R. Stat. Soc. A-135: 185–206.

Raina, A., J. Bland, M. Doolittle, A. Lax, R. Boopathy, and M. Folkins. 2007. Effect of orange oil extract on the formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 100: 880–885.

Rattan, R. S. 2010. Mechanism of action of insecticidal secondary metabolites of plant origin. Crop Prot. 29: 913–920.

R Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (http://www.R-project.org/).

Rust, M. K., and D. A. Reierson. 1998. Use of extreme temperatures in urban insect pest management, pp. 179–200. *In* G. J. Hallman and D. L. Denlinger (eds.), Temperature sensitivity in insects and application in integrated pest management. Westview Press, Boulder, CO.

Rust, M. K., and J. Venturina. 2009. Evaluation of chemical localized treatment for drywood termite control. *In* Progress report prepared for the California Structural Pest Control Board, Sacramento, CA, p. 31. (http://www.pestboard.ca.gov/howdoi/research/2009_drywood_rpt.pdf).

Scheffrahn, R. H., G. S. Wheeler, and N. Y. Su. 1997. Heat tolerance of structure-infesting drywood termites (Isoptera: Kalotermitidae) of Florida. Sociobiology 29: 237–246.

Warton, D. I., and F. K. Hui. 2011. The arcsine is asinine: the analysis of proportions in ecology. Ecology 92: 3–10.

Watanabe, Y., R. Mihara, T. Mitsunaga, and T. Yoshimura. 2005. Termite repellent sesquiterpenoids from *Callitris glaucophylla* heartwood. J. Wood Sci. 51: 514–519.

Wheeler, M. W., R. M. Park, and A. J. Bailer. 2009. Comparing median lethal concentration values using confidence interval overlap or ratio tests. Environ. Toxicol. Chem. 25: 1441–1444.

Wickham, H. 2016. ggplot2: elegant graphics for data analysis. Springer-Verlag, New York.

Xu, H., S. Qiang, P. Genovesi, H. Ding, J. Wu, J. Meng, Z. Han, J. Miao, B. Hu, J. Guo, *et al*. 2012. An inventory of invasive alien species in China. NeoBiota 15: 1–26.

Zhu, B. C., G. Henderson, F. Chen, H. Fei, and R. A. Laine. 2001. Evaluation of vetiver oil and seven insect-active essential oils against the Formosan subterranean termite. J. Chem. Ecol. 27: 1617–1625.