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Volatile Essential Oils Can Be Used to Improve the Efficacy of Heat Treatments Targeting the Western Drywood Termite: Evidence from Simulated Whole House Heat Treatment Trials

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Abstract

Colonies of western drywood termites, *Incisitermes minor* (Hagen) (Blattodea: Kalotermitidae), are difficult to detect and treat due to their cryptic nature. The use of heated air to create lethal temperatures within infested wood serves as a nonchemical treatment option targeting whole structure or large portions of the structure. However, the presence of hard-to-heat areas and potential risk of damage for heat-sensitive items are recognized as important challenges. Here, we tested if a localized injection of volatile essential oil could be utilized to address the heat sink issue, potentially increasing the overall efficiency of heat treatments against drywood termites. Artificially infested wooden blocks were placed in several locations of the test building, and heat treatments were conducted. For the treatment group, a small amount of essential oil (methyl salicylate) was added in the blocks prior to the heat treatment. All blocks placed in uninsulated wall voids had 92–100% termite mortality by day 7. However, the presence of a large concrete wall in the subarea hindered heating of blocks therein, resulting 36–44% mortality by day 7 when there was no essential oil treatment. Incorporation of the essential oil substantially increased the control efficacy for the subarea, resulting in more than 90% mortality. This approach might also be helpful in reducing the risk of potential heat damage during heat treatment without compromising its control efficacy.

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

The western drywood termite, *Incisitermes minor* (Hagen), is a common structural pest in the United States. It is native from northern Mexico to central California, and inland to the Sierras (Cabrera and Scheffrahn 2001). They cause significant structural damage across a wide area in the United States and Canada, particularly 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). Western drywood termites are economically important in California, with a previously estimated annual economic impact of $250 million (Cabrera and Scheffrahn 2001), a figure that has certainly increased since. Accordingly, there is an economic impetus to control *I. minor* in California and elsewhere.

Various control options are available in the event of a western drywood termite infestation. One common example involves injecting a liquid or dust insecticide through holes drilled into the infested wood to intersect termite galleries. Additionally, fumigation can be used to treat the whole structure by introducing gaseous toxicants (i.e., fumigants) to target all termite colonies inside.

For an alternative to fumigation, infested wood can be heated to eliminate drywood termite infestations if the wood is brought to a sufficient temperature for a long enough time (Lewis and Forschler 2014, and references therein). However, heat treatments may be impeded by structural features (heat sinks) that make it difficult to achieve the target temperatures necessary to eliminate all termites within certain pieces of wood (Lewis and Haverty 1996, Lewis and Forschler 2014).

The impact of heat sinks in whole-structure heat treatments for western drywood termite control has been previously demonstrated in an experimental structure (Lewis and Haverty 1996). Several wooden boards artificially infested with drywood termites were installed in various locations of an experimental structure, and the structure was subsequently heated with several propane heaters. Once thermocouples inserted into the wood showed a target temperature of 50°C, this temperature was maintained for 1 h. After the heat treatment, 100% mortality was achieved in all tested boards except for those positioned against a concrete foundation wall in the subarea of the structure (8.9% survival at week 4 posttreatment).

The findings suggested that the concrete foundation may have functioned as a structural heat sink and shielded some termites from being exposed to lethal temperatures. Higher target temperatures or longer heating times can be considered to solve for these 'worst case' scenarios where suspected infestations are thermally protected (Lewis and Haverty 1996, Scheffrahn et al. 1997). However, these approaches could increase the potential risk of damaging heat-sensitive items within the structure. The air temperature inside of heat-treated structures often reaches a level that is much higher than the target temperature. For example, Lewis and Haverty (1996) reported that the maximum air temperature in the test building reached >87°C during a heat treatment trial to achieve a target temperature of 50°C in all locations where temperatures were monitored.

To address this challenge, different strategies might be considered to selectively treat these 'hard-to-heat' areas of the structure to maximize the control efficacy of heat treatments. One option is to combine the heat treatment with a localized chemical treatment to target areas where heat is less effective. Though this approach would negate the nonchemical appeal of a heat treatment, incorporation of an active ingredient (AI) with relatively low mammalian toxicity and rapid dissipation in the environment might offset this loss. Previously, a combination of a whole-structure heat treatment and localized essential oil injections have been tested on a laboratory scale (Perry and Choe 2020). The authors placed individual termites within small wooden blocks designed to simulate infested wood, then subjected them to heat under various conditions. It was found that the addition of an artificial heat sink raised the temperature needed to attain complete mortality after 2 h of heating by several degree Celsius. However, adding a small amount of insecticidal essential oil resulted in the lowest temperature needed for complete kill. This study took place under highly controlled and simplified conditions, and a more realistic simulation of a combination heat and essential oil treatment is still needed.

Although various active ingredients might be considered for the combination treatment proposed here, essential oils insecticides have several characteristics that make them particularly suited for this purpose: 1) they are derived from 'natural' compounds, 2) some of them are on the FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act) section 25(B) list of pesticides exempt from registration requirements, 3) they are often quite volatile compared with other common synthetic AIs, and 4) they are typically repellent to many insect pests. Perry and Choe (2020) conducted a fumigant toxicity test using individual western drywood termites and several insecticidal essential oils, and found that the fastest kill was provided by methyl salicylate (MS).

Using the same experimental structure that was used by Lewis and Haverty (1996), the current study examined whether the incorporation of MS at the sites of heat sinks would improve the control efficacy of a subsequent heat treatment. First, wooden blocks were constructed from structural lumber to simulate infested wall studs or other structural boards. The blocks were placed in two zones within the test structure: within wall voids (no heat sink), and on the top of a concrete perimeter wall in the subarea (heat sink). MS was added to half of the blocks within each zone. Second, heat treatments were conducted in the experimental structure by licensed pest management professionals (PMPs), following the protocols that would be used in a typical heat treatment targeting drywood termites.

This study had two goals. The first goal was to verify whether the presence of structural heat sinks would shield termites and affect control efficacy in a 'real-life' heat treatment process, as was indicated by Lewis and Haverty (1996). It was hypothesized that control efficacy of the heat treatment would be significantly reduced with a heat sink present. The second goal was to determine whether the addition of MS at the sites of the heat sink prior to the heat treatment would significantly improve overall control efficacy. It was hypothesized that addition of MS would provide greater mortality of termites even when a heat sink is present.

Materials and Methods

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 diam., Millipore Sigma, Darmstadt, Germany). Balsa wood pieces were provided to serve both as food and 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.

Experimental Block

To simulate termite galleries in infested structural lumber, experimental blocks were constructed from pieces of Douglas fir. The wooden pieces used to form the blocks were 5.08 × 10.2 cm in height and width, respectively, and were purchased ~1.5 mo in advance of testing. For the interim, they were stored at 23–25°C and 34–45% relative humidity until used. The blocks (5.08 × 10.2 × 25.4 cm) were designed to house a group of western drywood termites during whole-structure heat treatments. Each block consisted of two pieces of wood (2.54-cm height each). To hold the termites, a narrow channel (0.7 × 1.35 × 20 cm, with both ends closed) was routed along the centerline of one piece from each pair. A small piece of cotton (43–47 mg) was placed at each end of the channel as the substrate for methyl salicylate application. A sheet of clear acrylic (0.2 × 10.2 × 25.4 cm, Plaskolite, Columbus, OH) was placed over the top of the channel-bearing bottom piece of the block to facilitate observation. The acrylic sheets were rinsed with 2% Liquinox detergent (Alconox, White Plains, NY) and deionized water prior to placement in an block to standardize the environment within. The top piece was placed on the bottom piece, with the acrylic sheet pressed between them, to form a complete block (Fig. 1).

In total, 20 pseudergates were added directly into the channel of each block before the heat treatment trials. Of these 20, at least one individual was an early-instar pseudergate (2- to 3-mm length), and at least one was a late-instar pseudergate with wing buds (7- to 9-mm length). The rest were intermediate-instar individuals without wing buds (4- to 6-mm length). Once the termites were placed in the channel, the two pieces of wood were placed together and secured using two heat resistant cable ties (Xtreme Ties, Gardner Bender, Menomonee Falls, WI). For some replications (4 out of 90 total blocks), a few termites were accidentally damaged and killed during the handling processes (e.g., when assembling the wooden blocks before heat treatment). These damaged termites were not included in the analyses. In these cases, the total number of termites was adjusted to calculate the percentage mortality.

In addition to the experimental blocks containing termites, six blocks were prepared for the purpose of monitoring the temperature

inside the channels during the heat treatment. These temperature monitoring blocks had a small hole (3.6-mm diam) drilled through the center of the channel and a thermocouple (0.63-mm copper-nickel, TEGAM Inc., Geneva, OH) was inserted in the hole. The tip

of the thermocouple was placed just above the floor of the channel, and a small amount of cotton was used to seal the gap between the hole and the thermocouple wire. Finally, a piece of packing tape was used to secure the cotton and thermocouple wire in place.

Fig. 1. Disassembled block showing the top (right) and bottom (left) pieces of block. A sheet of acrylic, the cotton balls, and termites inside the channel are shown on the bottom piece of the block (left).

Test Structure

The 'Villa Termiti', a full-sized experimental wooden structure located at the University of California, Berkeley Richmond Field Station (Richmond, CA), was used for the heat treatment trials (Fig. 2). This cubic building (6.1 × 6.1-m floor, with an area of 37.2 m^2 and a volume of 154 m^3) was previously constructed for the purpose of conducting experiments on wood destroying organisms. Four sides are constructed identically from Douglas fir studs and centers, with one door and two windows built into each wall, so that each side may be used as a replication. The structure includes an attic and a subarea with a concrete slab and perimeter foundation. Lewis and Haverty (1996) provided a detailed description and diagrams of the Villa Termiti.

Experimental Design

This project incorporated two experimental trials conducted on two consecutive days (16–17 October 2018) in the Villa Termiti using wooden blocks containing live drywood termites, distributed in two different areas (aboveground wall voids and subarea). Of the four walls in the structure, only the west, north, and east walls were used for trials due to a large joist preventing access to the south side of the subarea.

To simulate infested studs in the walls of a structure, the experimental blocks were placed in the aboveground wall voids (Fig. 3A). To access the wall voids, two sections of drywall touching the corners and extending ~0.9 m inward were cut open per side. To allow placement of blocks and ensure no heat sinks were present during treatment, the fiberglass insulation was partially removed from these sections of wall voids. Within each wall void, three experimental blocks were placed at heights of 80, 130, and 180 cm from the floor. Two long metal screws partially screwed into the wall at each height served as brackets on which the blocks were placed. The brackets

Fig. 2. 'Villa Termiti', the experimental structure used in the current study. (A) The south- and west-facing walls of the test structure before beginning treatment. (B) The south wall of the structure during the heat treatment, showing tarpaulins and a heater.

Fig. 3. Two different locations where the experimental blocks were placed. (A) Wall void. Brackets were used to place multiple blocks within a single wall void space. Four blocks are shown in the photo, and the bottommost one is a temperature monitoring block. (B) Subarea. The blocks were placed on the top of concrete perimeter wall. The leftmost block is a temperature monitoring block.

were each insulated with a folded piece of paper towel and tape. One temperature monitoring block was placed at a height of 25 cm in the left wall void of each wall. In total, 18 experimental blocks were placed in the wall voids.

To simulate an infested sill plate, the experimental blocks were placed on the top edges of the concrete perimeter walls in the subarea (Fig. 3B). As in the aboveground wall voids, the south wall of the subarea was not used. First, seven locations were marked on each perimeter wall, spaced 68-cm apart. After marking these locations, a horsehair brush was used to clean the top of the perimeter wall. To ensure thorough contact between the blocks and the perimeter wall surfaces, 250 g of sand was first placed on each location, and the block was placed on the top of the flattened sand patch. Thickness of the sand patches varied depending on the heterogeneity of the concrete surface beneath but was ~0.5 cm in most cases. Six experimental blocks and one temperature monitoring block (leftmost location) were placed on each perimeter wall. See Fig. 4 for the approximate location of each block in the test structure.

For both wall void and perimeter wall locations, half of the blocks were treated with MS (see Methyl Salicylate Treatment). Additionally, some blocks were kept in the laboratory at room temperature (23–27°C) as the no-heat controls (with or without MS). Therefore, the treatments were 1) heat/wall void/no MS, 2) heat/wall void/with MS, 3) heat/subarea/no MS, 4) heat/subarea/with MS, 5) no heat/no MS, and 6) no heat/with MS. Each treatment was replicated nine times per heat treatment trial (there were two separate heat treatment trials, see Heat Treatment). No-heat controls (5 and 6 above) were conducted only once. Altogether, 90 blocks were tested.

Methyl Salicylate Treatment

For the blocks that were assigned to MS treatments, 80 µl of MS (99%, Sigma–Aldrich, St. Louis, MO) was added to each of two cotton balls in the channel (160 µl per block) using a 100-µl glass syringe (Gastight syringe, Hamilton Company, Reno, NV). The amount of MS to be added was determined by taking the ratio of the volumes of laboratory-scale channels (see Perry and Choe 2020) and that of the current study. The application of MS was made immediately prior to placing the blocks in the test structure. To minimize cross contamination with MS volatiles between different blocks/
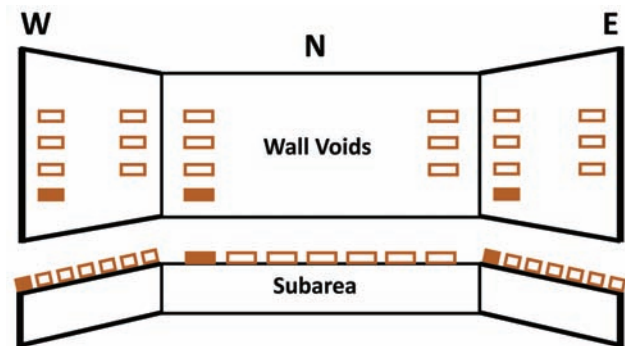

Fig. 4. A schematic diagram showing the relative positions of the blocks (total 42 blocks are shown) in the wall voids and subareas of the west, north, and south walls. Filled-in blocks denote temperature monitoring blocks. Not drawn to scale.

treatments, particularly in the subarea where blocks were placed in close proximity, the gap between the two wooden pieces in a block was sealed by wrapping the side of the block (including the wood–acrylic–wood interface) with duct tape.

Heat Treatment

Whole-structure heat treatment of the Villa Termiti was conducted by Greentech Heat Solutions (Anaheim, CA). First, tarpaulins were hung from the eaves of the test structure and secured using clamps and sandbags. Heated air was provided with two 990,000 BTU propane heaters and one 440,000 BTU heater (Greentech Heat Solutions). Ducting was used to carry and direct the heated air within the structure. Three heaters and attached routes of ducting were used: the first route entered through the north door of the structure and blew directly downward through a hatch into the subarea, the second route entered through the south door and emptied into the main space, and the third route remained outside the structure and heated the outside walls beneath the tarpaulins. Additionally, two box fans (AM 4,000 CFM, Greentech Heat Solutions) were placed in the main space of the Villa Termiti to provide air flow and reduce the incidence of heat stratification.

According to Forbes and Ebeling (1987), exposure to 48.9°C for 30 min is sufficient to kill drywood termites. Additional research

has indicated that 10- to 15-min exposure to temperatures around 50°C is sufficient to kill several economically important species of drywood termites (Scheffrahn et al. 1997). Based on these studies, a target temperature of 49°C (inside of the wooden block) was chosen for both treatments. The temperature measured within each temperature monitoring block was recorded at least once every 30 min. Two separate heat treatment trials were conducted on consecutive days, with the structure left to cool overnight. For trial 1, once all three temperature monitoring blocks in the aboveground wall voids reached the target temperature (49°C), heating was continued for one additional hour (heat applied for a total of 140 min). This simulated a standard heat treatment protocol. However, 30 min prior to the end of the trial, temperatures recorded within the subarea blocks began to approach the target temperature for the wall voids. To avoid achieving a level of heat in the subarea that might render the results uninformative, the heater that blew into the subarea was turned off 30 min prior to the other heaters (see Discussion). For trial 2, the heaters were shut off immediately once the target temperature (49°C) was reached in all three wall voids (heat applied for a total of 126 min). This simulated a suboptimal, short protocol for heat treatment. In both cases, the structure was opened and allowed to cool for 10 min before the blocks were retrieved.

For trial 1, in addition to the temperatures inside of the wooden blocks, the air temperature in the test structure during the heat treatment was recorded using two data loggers (Hobo data loggers, Onset Computer Corporation, Bourne, MA). The first data logger was placed atop the temperature monitoring block in the north wall. The second data logger was placed on the leftmost end of the north perimeter wall in the subarea.

Data Collection

Once the blocks were brought back to the laboratory, they were placed on a counter in separate piles for wall void, subarea, and no-heat control blocks to minimize any cross contamination. Each block was then opened, and forceps were used to transfer the termites to a plastic petri dish (60 × 15 mm, 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. Immediately after the conclusion of treatment and once a day for 14 d afterward, the number of dead or moribund termites was counted for each block based on the behavioral response of termites when probed with forceps. For the purposes of the current experiment, 'mortality' included both dead and moribund termites. Death was characterized by no response to light prodding with forceps. Moribundity was characterized by weakened or spastic leg movements and inability to grip onto the substrate. Separate pairs of forceps were used for different treatment groups to minimize cross contamination.

Data Analyses

All statistical analyses were carried out using R version 3.4.1 (R Core Team 2013). To illustrate the changes in temperature within each area of the test structure, as well as within the blocks themselves, data from the temperature monitoring blocks were graphed for the subarea and wall voids, and in the case of trial 1 the temperature data recorded by the Hobo data loggers as well (Wickham 2016). Since three temperature monitoring blocks were placed in each area of the structure (one for each side), the average recorded temperatures at each timepoint were used.

For each treatment, overall cumulative mortality data were graphed for days 1–14 posttreatment to illustrate trends in mortality

over time (Wickham 2016). The pooled data from nine replications (e.g., % mortality of 180 termites) were used for each treatment. 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 (see Results).

To determine whether the mortality caused by the different treatments was significantly different, nonparametric statistical tests were conducted. First, a Kruskal–Wallis one-way analysis of variance was used to compare the % mortality data from the heat treatments for each trial (R Core Team 2013). Once the null hypothesis was rejected (i.e., at least one population median of one group is different from the population median of at least one other group), Dunn's multiple comparisons were used as a post-hoc analysis to determine whether % mortality significantly differed between individual treatments. Specifically, the wall void and subarea treatments with heat only were compared, as well as treatments with and without MS in the subarea location (Ogle et al. 2019). A Wilcoxon rank-sum test was used to compare no-heat controls to determine whether the presence of MS significantly affected % mortality in the absence of heat treatment (R Core Team 2013).

Survival times were analyzed using a Kaplan–Meier survival analysis (Kassambara and Kosinski 2018). This analysis compared the distribution of survival times using the survivorship function S (t), the probability of an individual termite surviving past a given timepoint t. Multiple comparisons of the survival curves were conducted using log-rank tests with BH-adjusted *P*-values (Mantel 1966, Peto and Peto 1972, Kassambara and Kosinski 2018). Particular attention was paid to whether mortality trends differed between wall voids and the subarea with the absence of MS (i.e., 'heat/wall void/ no MS' vs 'heat/subarea/no MS'), and between treatments in the subarea with and without MS (i.e., 'heat/subarea/no MS' vs 'heat/ subarea/with MS').

Results

Temperature Trends

Trial 1 temperature data for the data loggers, as well as averaged data from the temperature monitoring blocks, are summarized in Fig. 5. Before the heat treatment (trial 1), the ambient temperatures recorded in the wall void and subarea were 25.1 and 29.8°C, respectively. During the heat treatment, the peak temperatures recorded in the wall void and subarea were 57.0 and 63.6°C, respectively. The average temperature within the wall void blocks closely matched the air temperature in the wall void during the treatment. In contrast, the air temperature in the subarea remained higher than the average temperature within subarea blocks, and the highest recorded temperature (63.6°C) was from the data logger in the subarea. The greatest difference in temperatures between the interior and exterior of the wall void blocks (11.7°C) was recorded at the start of the trial. The greatest difference in temperature between the interior and exterior of the subarea blocks (22.9°C) was recorded 8 min prior to turning off the heater that blew directly into the subarea.

Heat Treatments

Overall cumulative mortality data from both trials and the no-heat controls are summarized in Fig. 6. Across all treatments, some of the 1 d survivors were affected by the treatment, and mortality increased in the subsequent observations. Cumulative mortality by 7 d accounted for 97% of the total mortality (14 d posttreatment mortality). Additionally, in the no-heat, no-MS control trials, mortality rose from 3.8% by 7 d to 8.8% by 14
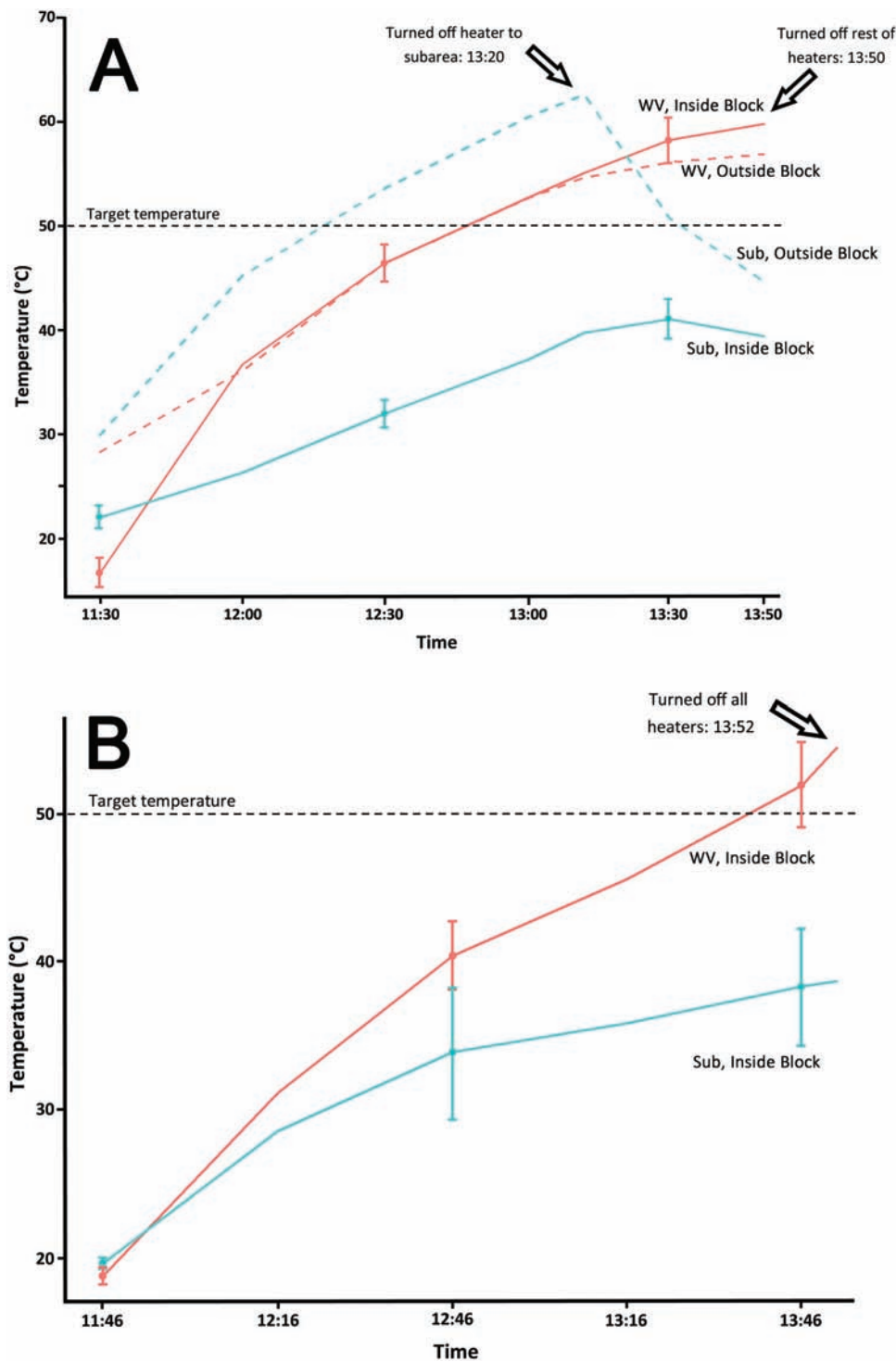
Fig. 5. Temperature measurements both within temperature monitoring blocks using thermocouples, and outside blocks using data loggers. Lines for temperature monitoring blocks represent the average temperatures from three blocks, and the SEM is indicated at 0, 1, and 2 h after beginning treatment. 'WV' indicates wall void, and 'Sub' indicates subarea. (A) Trial 1. Data from inside and outside blocks are shown. (B) Trial 2. Only data from inside blocks are shown.

d. Background mortality close to 10% could substantially affect the data analyses and interpretation. For these reasons, the cumulative mortality data up to 7 d posttreatment were used for the subsequent statistical analyses.

Mortality data for both trials as well as the controls are summarized in Table 1. Regardless of the presence or absence of MS, all treatments in wall voids achieved 92–100% mortality. In fact, all replications in the

wall voids had 100% mortality except one block in 'no MS' treatment which had 70% survivorship. In contrast, the presence or absence of MS was a significant factor for the mortality in subarea treatments. For example, the mortality from subarea treatments without MS averaged 44 and 36% for trial 1 and 2, respectively. However, subarea treatments with MS had >90% mortality. No-heat controls averaged 4% mortality without MS, and 72% with MS.
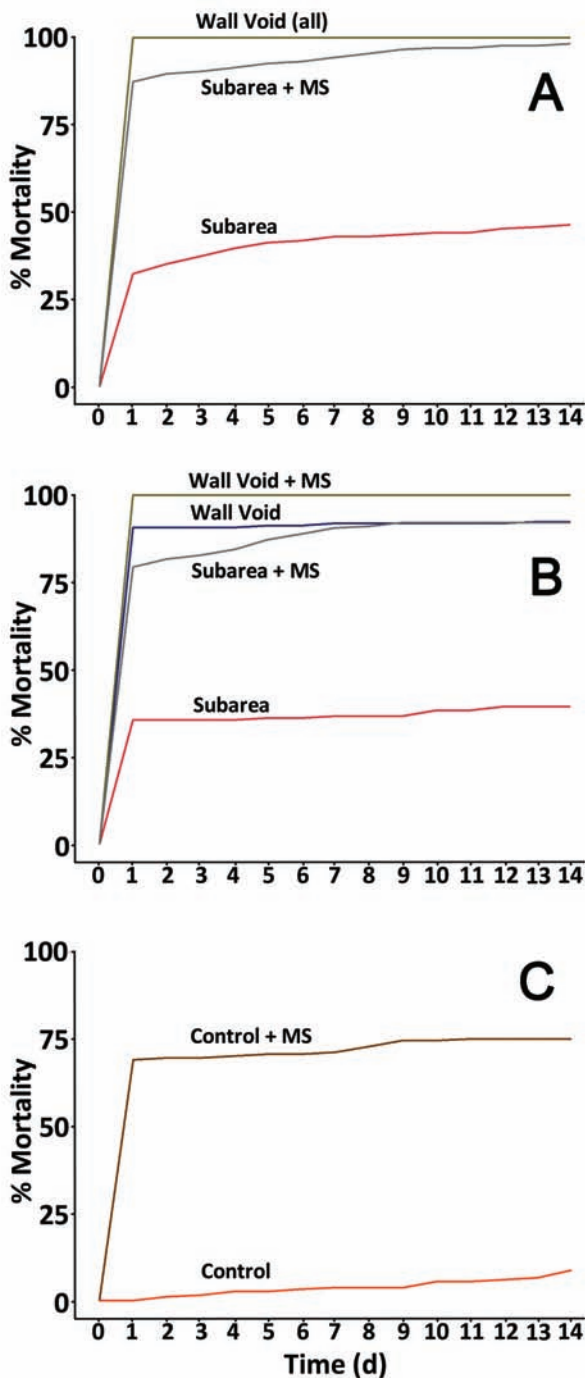
Fig. 6. Overall cumulative % mortality from pooled data (of 180 termites for each treatment or control). (A) Mortality from heat treatment trial 1. Mortality lines from 'wall void' and 'wall void + MS' overlap. (B) Mortality from heat treatment trial 2. (C) Mortality from no-heat controls.

Nonparametric Tests

A Kruskal–Wallis test showed that there was a significant overall difference in mortality among the treatments within trial 1 ($H = 20.19$, df = 3, $P < 0.001$) as well as within trial 2 ($H = 13.60$, df = 3, $P = 0.0035$) (Table 1). From trial 1, Dunn's multiple comparisons revealed that 'heat/subarea/no MS' had significantly lower mortality than all other treatments ($\alpha = 0.05$), among which there were no significant differences. Multiple comparisons for trial 2 found

Table 1. Western drywood termite mortality across all treatment conditions and trials at the test structure

Heat treatment trial No.	Treatment			Mortality (%) at 7 d[a] (mean ± SEM)
	Location	MS	n	
1	Wall Void	–	9	100a
		+	9	100a
	Subarea	–	9	44 ± 13b
		+	9	93 ± 6a
2	Wall Void	–	9	92 ± 8a
		+	9	100a
	Subarea	–	9	36 ± 16b
		+	9	90 ± 6a
No-heat control	Laboratory	–	9	4 ± 2a
		+	9	72 ± 13b

[a]Values for % mortality represent the percentage of 20 individual pseudergates ($n = 9$). Within a trial, values with a same letter are not significantly different at $\alpha = 0.05$ (Dunn's multiple comparison and Wilcoxon rank-sum test for heat treatment trials and no-heat controls, respectively).

that 'heat/subarea/no MS' was significantly less effective in killing termites than both treatments (with or without MS) in wall void ($\alpha = 0.05$), with no other significant differences between treatments detected in the wall voids or subarea. Additionally, a Wilcoxon rank-sum test indicated that 'no heat/with MS' killed more termites than 'no heat/no MS' ($P = 0.0031$).

Survival Analyses

Results from the Kaplan–Meier survival analyses for the heat treatment trials and no-heat controls are shown in Fig. 7. Global log rank tests indicated that there is a significant difference among the survival curves for both trial 1 ($\chi^2 = 362$, df = 3, $P < 0.001$) and trial 2 ($\chi^2 = 284$, df = 3, $P < 0.001$).

First, pairwise comparisons of the survivorship curves revealed that survival times were significantly different between 'wall void' and 'subarea' when MS was not used (log-rank test: $P < 0.001$ for both trials) (Fig. 7A and B). Survivorship in 'wall void/no MS' quickly dropped to 0 (trial 1), or a very low level (~0.1) (trial 2) and remained low until day 7 posttreatment. However, survivorship in 'subarea/no MS' was ~0.6 to 0.65 by day 7 posttreatment in both trials, indicating >60% of termites survived the heat treatment. Overall, these results provided strong evidence that the presence of the heat sink in the subarea increased survivorship of termites.

Second, treatment with MS had a significant impact on termite survivorship for the blocks placed in the subarea. Pairwise comparisons of the survivorship curves revealed that survival times were significantly different between 'subarea/no MS' and 'subarea/with MS' (log-rank test: $P < 0.001$ for both trials). While > 60% of termites were still alive by day 7 posttreatment in 'subarea/no MS', survivorship in 'subarea/with MS' quickly dropped to low levels (~0.15 to 0.2) by day 1 posttreatment and remained low (Fig. 7C and D). Overall data suggested that treatment with MS provided a quick kill despite the presence of a heat sink.

Finally, the survivorship analysis indicated that survival times were different between 'no heat/MS' and 'no heat/no MS' (Fig. 7E, log-rank test: $P < 0.001$). Without MS added, survivorship remained close to 1.00. However, the addition of MS resulted in an immediate drop in survivorship to ~0.30 due to much higher initial mortality, with a slight, gradual increase over the observation period.
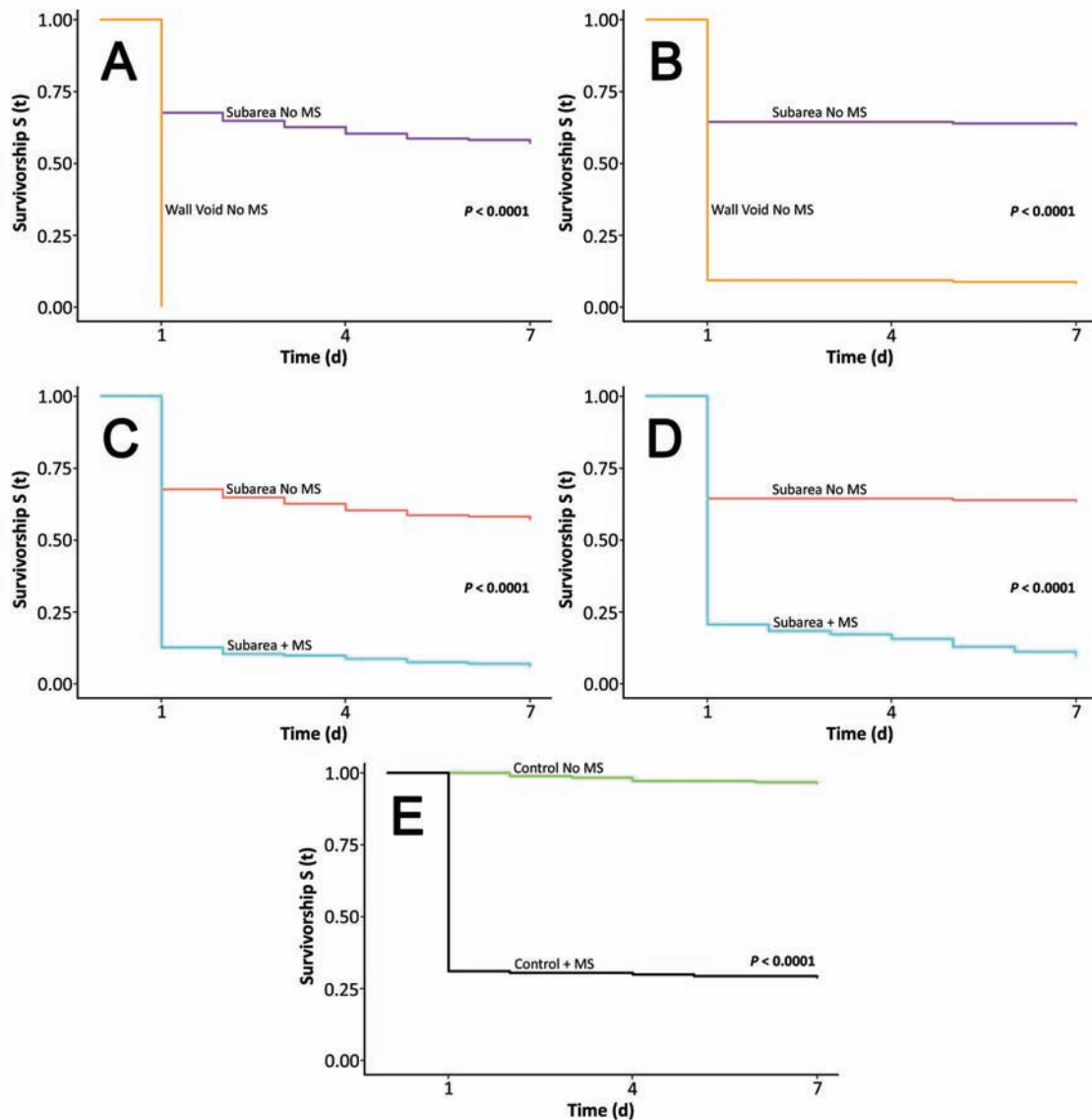
Fig. 7. Survivorship curves for treatments within trials and controls. Each interval represents the probability of an individual termite surviving until a given day (1–7) during the observation period. (A) Wall void and subarea treatments (trial 1), both without MS. (B) Wall void and subarea treatments (trial 2), both without MS. (C) Subarea treatments (trial 1), with and without MS. (D) Subarea treatments (trial 2), with and without MS. (E) No-heat controls, with and without MS.

This indicated that even in the absence of a heat treatment, fumigant and/or contact action from MS can cause substantial mortality of the drywood termites.

Discussion

Overall cumulative mortality data over time indicated that mortality observed at 1 d posttreatment was not always indicative of final mortality. For example, in some treatments of the subarea (with or without MS), mortality continued to accrue after 1 d posttreatment. This may have been caused by residual effects of exposure to heat, especially considering that the peak temperature in the subarea was higher than in the wall voids. For these reasons, mortality data up to 7 d posttreatment were chosen for subsequent statistical analyses.

Blocks located in the wall voids did not directly contact any surfaces other than the screws they rested on and were not shielded

to any significant degree by insulation. Thus, termites placed in the wall void were predicted to experience high/complete mortality after the heat treatment. In contrast, the presence of the heat sink (i.e., concrete perimeter wall) in the subarea was predicted to significantly reduce the efficacy of heat treatment. Observed differences in 7-d mortality between wall void and subarea treatments (both without MS) were consistent with the predictions. Mortality was significantly higher in 'heat/wall void/no MS' than in 'heat/subarea/no MS', indicating that the perimeter wall acted as an effective heat sink and shielded the termites from the heat treatment. The observed temperature discrepancy between 'inside block' and 'surrounding air' in the subarea (i.e., lower temperatures recorded within the blocks when compared with that of the surrounding air) also corroborated the 'heat sink' effect in the subarea.

Adding MS to blocks in the wall voids did not change mortality; 'heat/wall void/no MS' and 'heat/wall void/with MS' both had 100% mortality. This indicates that heat by itself was sufficient

to provide control in the wall voids, which is consistent with the fact that the blocks in this area had no significant sources of insulation nearby. However, the addition of MS to blocks in the subarea treatment (heat/subarea/with MS) significantly increased mortality to a level that was similar to the treatments in the wall voids. This finding indicates that localized injection of MS at the sites of heat sinks mitigates their effects, improving overall heat treatment efficacy.

Ideally, an effective treatment for drywood termites must eliminate all individuals from the infested structure, because just a few surviving termites may be able to recover and develop neotenic reproductives (Smith 1995, Lewis et al. 2009). Even though the current study clearly demonstrated that addition of methyl salicylate at the sites of heat sinks significantly improved the efficacy of a heat treatment, complete control was not provided in subareas, indicating that further improvement may be necessary. First, this might be due to the fact that only 160 μl of methyl salicylate were added per block, which would likely be less than in a real structural treatment. Second, out of nine blocks in each MS treatment, survival mostly occurred in one block from trial 1 (42.9% mortality), and two blocks from trial 2 (68.4 and 50% mortality). Average mortality among the remaining blocks was 98.7% (trial 1) and 99.3% (trial 2), so it is possible that other factors (i.e., incomplete circulation of heated air) resulted in high survival rates within specific blocks. Third, in trial 1, the heater that was positioned to blow into the subarea was turned off 20 min prior to the end of the treatment. This modification to the protocol was made in the interest of preventing the blocks in the subarea from reaching such a high temperature that overall mortality would be too high for the results to be informative. In doing so, some realism of the treatment was sacrificed. However, in a real treatment, it is unlikely that PMPs would have detailed knowledge of the locations of infested wood and would not have the ability to bring heated air to the specific locations as precisely as in this study. Finally, it should also be noted that the wooden blocks were removed from heat and brought to ambient temperature (24–26°C) immediately following the heat treatment process. In an actual structural heat treatment for drywood termites, the infested wood remains within the heated structure even after the heating process ends. Thus, in structural heat treatments, the exposure time to high temperatures is likely to be longer compared to what was simulated in the current experiment. If this is true, the findings from the current study would provide a more conservative estimate of termite mortality than a real treatment.

Compared with fumigation, one of the important advantages of heat treatment for drywood termite control is its relatively short treatment time. For a heat treatment, the structure needs to be vacated only for <1 d (Forbes and Ebeling 1987, Ebeling 1994), and in fact, the two heat treatments conducted in the current study both had durations of <3 h. Conducting an inspection for difficult-to-heat areas (close to heat sinks) and injecting insecticidal essential oil constituents prior to heat treatment might increase the time required for treatment. However, addressing the effects of heat sinks reduces the target temperature or length of treatment time needed for the heat treatment. For instance, in homes with concrete foundations, the sill plate is usually the last area to reach target temperature during a heat treatment (Ebeling and Forbes 1988, Rust and Reierson 1998). In such cases, targeted use of essential oil constituents in the sill plate prior to the heat treatment might effectively address the termite infestations in this location without major increase in overall treatment time.

There are several points that need to be considered in translating the current findings to practical application. First, it should be noted that the current experimental design incorporated direct application of methyl salicylate at both ends of a long and narrow gallery. Considering their contact toxicity (D. T. Perry, unpublished data, Tripathi et al. 2009, and references therein), methyl salicylate and other essential oil compounds are expected to kill termites by direct contact (i.e., when the oils are applied directly on the insects, or when the termite gallery is inundated with injected essential oil). However, the current experiment was designed conservatively to determine whether a small amount of the essential oil within the termite gallery (i.e., part of the gallery wall has received methyl salicylate from a nearby injection site) could still be effective in improving heat treatment efficacy. Based on the properties of these essential oil compounds and other preliminary observations, it would be reasonable to assume that essential oil injected into the wood would seep or diffuse into the wood and travel some distance from the initial injection sites. Although methyl salicylate may have traveled directly through the channel in the experimental block, the utilization of a piece of cotton as an injection point minimized this issue. In a real-world scenario, the holes for injecting the oil would need to be closely spaced so as to ensure that any termites present in the wood are exposed to the oil or its volatiles. To avoid drastically increasing the labor and costs associated with treatment, only wood that is shielded by heat sinks should be considered for drilling.

Another potential obstacle is the presence of residual odors of essential oils. Insecticidal constituents of many botanical essential oils, such as wintergreen oil and orange oil, tend to have strong odors, which may be further enhanced at high temperatures. It is possible that the holes drilled into infested wood in a real treatment would allow the odors to permeate treated structures. Although MS has relatively low inhalation toxicity (Gage 1970), additional aeration might be necessary to remove essential oil volatiles from the living spaces before reentry. This issue could be avoided by modifying the protocol for the treatment. For instance, if wood were only treated with the essential oil constituent from outside the structure, or within a crawlspace or attic, the presence of strong odors in the living spaces could be minimized. Additionally, to better suit customers who dislike or are sensitive to these odors, other essential oil compounds/constituents with similar insecticidal activity but with less odor could be tested in conjunction with heat treatment. For example, (–)-isopulegol was repellent to stored product pests and has a relatively low scent profile (Shimomura et al. 2018). Based on our preliminary test, (–)-isopulegol volatiles resulted in 100% mortality of five western drywood termites within 4 h (D. T. Perry, unpublished data). The use of less odoriferous essential oils warrants further investigation.

The current study clearly demonstrated that a typical heat treatment process will not be able to provide complete control of drywood termites if the termite colonies are situated in close proximity to a structural heat sink. However, localized application of small amounts of volatile essential oil within termite galleries was effective in addressing this issue, dramatically increasing overall mortality of drywood termites even for the locations with heat sinks. The overall results of the current study are corroborated by the findings of previous laboratory-scale experiments (Perry and Choe 2020). Since direct access to infested wood can be difficult or impossible in some situations, the combination of whole-structure heat treatment with localized injections of insecticidal essential oil constituents may not be feasible for all treatment scenarios. However, for structures in which wood adjoining structural heat sinks is accessible, this treatment combination may provide quicker and more thorough control than a stand-alone heat treatment. It is important to

point out that this approach combines heat and chemical (with fumigant action) treatments so that it does not qualify as a nonchemical treatment option. Nevertheless, the improved control efficacy of this combination approach might help to reduce the incidence of callbacks for PMPs. Ultimately, this may also help to improve the viability of heat treatments for drywood termite control by addressing concerns on possible heat damage due to long heating time or use of excessive temperatures (Lewis and Haverty 1996, Hammond 2015).

Acknowledgments

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