Title
The Use of an Essential Oil Adjuvant to Improve the Efficacy of Heat Treatments for the Western Drywood Termite

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The Use of an Essential Oil Adjuvant to Improve the Efficacy of Heat Treatments Targeting the Western Drywood Termite

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DEDICATION

To all the termites that gave their lives for this research.

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Chapter I. Introduction

The western drywood termite, *Incisitermes minor* (Hagen), 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). In their natural habitats, colonies of western drywood termites typically reside in dry, dead portions of various trees, such as eucalyptus, oaks, citrus, cottonwood, ash, walnut, and cypress (Hunt 1949, 1966; Snyder 1954). However, with the advent of global commerce and extensive shipping of structural lumber, western drywood termites have been provided with abundant food sources and long-distance transportation to previously unaffected areas. Currently, the western drywood termite is recognized as one of the major pests affecting human-made wooden structures in North America. Western drywood termites are pests in their native range and have become established elsewhere in the U.S. and Canada, and they are particularly problematic in California and Florida (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 economic impact of $250 million (Cabrera and Scheffrahn 2001), a figure which certainly has increased in the intervening years. Thus, detection and control of this pest are of importance in California and other regions in which *I. minor* has become established.

Western drywood termites are single-piece nesters; once a colony is established by a pair of winged reproductives (alates) in a piece of wood, individuals of the colony will stay within that piece of wood except for occasional / periodical dispersal by the new alates during mating season (Harvey 1934). The winged alates swarm (typically between early summer and late fall depending on location), form mated pairs, and burrow into pieces of dead wood to begin new colonies (Harvey 1934, Nutting 1969, Weesner 1970). Although many nests can be established simultaneously through this swarming process, the reproduction rate and growth of western drywood termite colonies are relatively slow. For example, a typical western drywood termite colony will only reach populations of 75-700 termites after four years, possibly with its first set of alates by then (Harvey 1934). This slow colony growth makes western drywood termite colonies difficult to detect until they establish and reach certain sizes.

One of the common methods for determining the presence of drywood termite infestations is visual detection of emerging alates (e.g., alates themselves, presence of shed wings), and/or the buildup of frass pellets as they are ejected from kickout holes in the infested wooden substrate (Harvey 1934, Hunt 1966). Even though the presence of emerging alates would be an unequivocal indication of live drywood termite activity, alate emergence only occurs during periodic mating swarms, i.e., observers must be present when swarming occurs. In contrast, frass that accumulates outside of kickout holes is available for visual detection at any time point, providing an importance piece of indirect evidence for drywood termite infestation (either current or previous infestation). If an infestation is suspected, wood members are commonly probed to determine whether galleries are present beneath the surface (Su and Scheffrahn 2000, Lewis and Forschler 2014). However, many of the wooden members in a structure might be inaccessible, providing a challenge to achieving a thorough inspection using visual or physical methods only (Lewis and Forschler 2014). Besides these direct visual inspection methods, there are various types of technology for detection of drywood termites, including x-ray, microwave, acoustic emissions, or infrared radiation to penetrate infested wood and detect the bodies of termites, their vibrations or body heat. However, none of these have been shown to reliably detect the presence of termites in all areas of a structure, given the presence of obstacles such as wall coverings (Lewis 2009). Due to their lack of cost-effectiveness, many of these devices are not used by pest management professionals (PMPs).

If an infestation is confirmed, several control options are available for western drywood termites. The control methods employed by PMPs can be broadly grouped into two categories: localized treatment and whole structure treatment (Scheffrahn and Su 1994, Lewis and Haverty 1996a, Lewis 1997). Localized or “spot” treatments involve a targeted application of insecticidal agents (either chemical or non-chemical) to eliminate relatively localized / well-defined colonies. The most common method of localized treatments is a liquid or dust insecticide injection, introduced via holes drilled into the infested wood to intersect termite galleries. Ideally, the injection utilizes a compound that can be readily transferred between individuals within the colony to maximize its impact (Hunt 1949, Lewis 1997). In the past, other methods for localized treatments included freezing the infested wood using liquid nitrogen (Forbes and Ebeling 1986), sending high-voltage electricity through the infested wood (Ebeling 1983), or using microwaves to heat the termites within the wood (Lewis and Haverty 1996a). The equipment for the treatments was expensive and not cost-effective. For these reasons, none of these treatments have been widely adopted by PMPs.

Whole structure treatments simultaneously affect all termites in the structure, with the goal of eliminating all termite colonies that may be present. Compared with localized treatment options, whole structure treatment methods tend to be more time-consuming and expensive (Scheffrahn and Su 1994). However, whole structure treatment methods provide a greater likelihood of achieving complete control, especially when there is a chance that some colonies remain undetected and untreated. If all termites are not eliminated from the structure, there is a possibility for the colonies to recover due to the ability to replace primary reproductives with supplementary reproductives (Harvey 1934, Smith 1995). Therefore, when properly conducted, whole-structure treatments may result in fewer callbacks (which may require retreatment) for PMPs (Ebeling and Wagner 1964, Scheffrahn and Su 1994).

Fumigation is one of the methods targeting the whole structure for western drywood termite control. Fumigation utilizes gaseous toxicants (i.e., fumigants) to eliminate all termite colonies within the structure. The efficacy of fumigation is a function of the exposure time and dose of fumigants. For instance, Stewart (1957) conducted laboratory trials in which immature western drywood termites were exposed to sulfuryl fluoride or methyl bromide under various conditions. For both compounds, a concentration of $\approx$ 20 mg/L was necessary to achieve almost complete mortality if the insects were exposed for 4 h, while only $\approx$ 2.75 mg/L was necessary if exposure lasted for 24 h (Stewart 1957). Sulfuryl fluoride is the only fumigant that is currently registered for use in structural pest control worldwide (Lewis and Forschler 2014, Gillenwaters and Scheffrahn 2018).

A properly conducted fumigation will eliminate all drywood termites from a structure (Stewart 1957, Osbrink *et al*. 1987, Scheffrahn and Su 1992, Su and Scheffrahn 2000). Consequently, fumigation is often selected when structures are involved in real estate transactions. In spite of its effectiveness in treating extensive drywood termite infestations, fumigation has some drawbacks. For example, the entire process (e.g., setting up the fumigation tarpaulin, introduction of the fumigant, aeration) takes several days. During this period, the structure must be vacated. Besides concerns regarding its high mammalian toxicity (Eisenbrandt and Hotchkiss 2010), sulfuryl fluoride is a potent greenhouse gas, with an atmospheric lifetime of up to 36 years (the amount of time required for the released sulfuryl fluoride to return to a natural level via reaction or a sink) (Tsai 2010). According to the California Department of Pesticide Regulation, ~ 1,256 metric tons of sulfuryl fluoride were used for structural pest control in California in 2016 (DPR 2016), most of which was likely used for drywood termite fumigation. Finally, fumigation has no residual activity once the treatment is concluded, so residual protection against new drywood termite infestation is not provided (Hunt 1949).

Another method to target the whole structure for western drywood termite control is heat treatment. Depending upon the size and configuration of the structure, a heat treatment can be used to treat the entire structure or certain sections of it. The use of high temperatures to control drywood termites was first reported by Ebeling (1975). High temperatures have also been shown to be effective for the control of other pests such as cockroaches and fleas (Grahl 1990, Ebeling 1994). The goal of a heat treatment is to bring the core temperature of structural wood to a level that is lethal to the termites (Ebeling 1994). Several experiments have reported that exposure to 48.9 - 49 °C for > 1 h (Forbes and Ebeling 1987, Lewis and Haverty 1996a), or exposure to > 49 °C for 30 min is lethal to the western drywood termite (Ebeling and Forbes 1988, Rust and Reierson 1998). Laboratory and field experiments found that another economically important drywood termite species, *Cryptotermes brevis* (Walker), could be controlled by raising the core temperature of infested wood to 49 °C and subsequently maintaining it for 1 h (Woodrow and Grace 1998a, b). Scheffrahn *et al*. (1997) concluded that achieving an internal wood temperature of 54.4 °C for 1 h will result in complete mortality for most pest drywood termite species inside the wood.

To conduct a heat treatment, heaters (propane- or electricity-powered) are used to blow heated air into the structure, often with several additional fans to circulate the heated air throughout the structure (Lewis and Forschler 2014). Additional heaters and fans may be used to circulate heated air against the outside of the structure so that the walls are heated from both sides. To ensure the target temperature is reached, the internal temperatures of the wood members are monitored with thermocouples inserted into the wood. Tarpaulins are draped around the structure to contain the heated air, which also helps to heat the walls from both sides (Forbes and Ebeling 1987, Ebeling 1994). However, unlike fumigation, heat treatment does not require a complete sealing of the structure with tarpaulins. In fact, some amount of air escapes through the openings to aid air circulation (Forbes and Ebeling 1987, Lewis and Haverty 1996b). As the heated air rises within the structure, heat is transferred into the attic and is dispersed through the roof. Since alates of western drywood termites tend to enter structures via vents and other openings in attics (Snyder 1954, Ebeling 1975, Scheffrahn and Su 1994, Smith 1995), the heated air rising and escaping through the top portion of the structure may help to treat this high-risk area.

Similar to fumigation, heat treatment does not provide residual protection from future infestation by new termites. However, compared to fumigation, heat treatment for drywood termite control has several advantages. First, heat treatment does not rely on any chemical toxicant to control the termites (Ogle 1990, Ebeling 1994). Secondly, achieving lethal temperatures in a structure takes a relatively short amount of time and only requires the structure to be vacated for less than a day (Forbes and Ebeling 1987, Ogle 1990). Lastly, a heat treatment can be scaled down and adapted to treat certain subsections of the structure (e.g., one room, certain walls, the attic) in situations where the whole structure does not need to be treated (Ebeling 1994). In these scenarios, only part of the structure needs to be prepared and vacated during the treatment, providing a greater level of flexibility compared to fumigation.

The use of heat treatment for drywood termite control poses some challenges. First, the high temperatures maintained within the structure might damage some heat-sensitive items in the structure. Thus, plastics and electronic items need to be moved or covered with insulating blankets prior to the heat treatment (Ogle 1990, Rust and Reierson 1998). The extreme temperature and drying power of the heated air can also result in cosmetic damage in some structural items such as wooden doors or floor materials (Lewis and Haverty 1996a, Hammond 2015). Secondly, some sections of the structure may not reach the target temperature due to heat stratification or the presence of structural "heat sinks". Heat stratification may occur if the heated air is not evenly distributed through the structure, but adequate use of fans will minimize this problem (Ebeling and Forbes 1988). Structural heat sinks may be provided by any structural features (e.g., wood contacting concrete walls or foundation) that potentially allow heat to dissipate quickly (Lewis and Haverty 1996a, Rust and Reierson 1998, Lewis and Forschler 2014).

Laboratory observations indicate that western drywood termites avoid high temperatures by moving to cooler sections of their galleries (Cabrera and Rust 1996, Rust and Reierson 1998, Cabrera and Rust 2000). Therefore, the presence of heat sinks in a structure might allow some termites to avoid exposure to lethal temperatures if their galleries extend to these "hard-to-heat" areas. The surviving colonies of drywood termites may result in callbacks for the PMP and inconvenience for homeowners. Continuing the heating process until all structural lumber reaches the target temperature is one possible solution. Rust and Reierson (1998) proposed that 6 h of heating would be sufficient for most homes. However, this guideline may not be appropriate for all scenarios, and some structures might take significantly longer than others to fully heat. For example, increasing the heating time increases the treatment cost, the time period during which the structure needs to be vacated, and the potential for heat damage to household / structural items.

Due to the aforementioned reasons, the efficacy and economics of heat treatment could be significantly improved if the effects of structural heat sinks were mitigated. One of the possible methods to achieve this would be injecting an insecticide at the site of structural heat sinks, thus killing drywood termites that attempt to take refuge in these areas. However, incorporation of synthetic insecticides into the heat treatment protocol would void the nonchemical nature of heat treatment, possibly making it less practical due to regulatory and/or marketability issues (i.e., additional licenses may be required for PMPs, and the method will be less attractive for customers with a negative perception of synthetic insecticides). On the other hand, some botanical essential oils and their constituents with insecticidal properties (Shaaya *et al.* 1991, Askar *et al.* 2016) might be considered for this particular use. Most botanical essential oils and their constituents have low mammalian toxicity (Regnault-Roger *et al*. 2012), and many are exempt from United States Environmental Protection Agency registration under section 25(b) of the Federal Insecticide Fungicide and Rodenticide Act (EPA 1996). The use of essential oils and their constituents as insecticides began long ago, and research into their potential for controlling different pest insects is an ongoing process (Zhu *et al.* 2001, Dayan *et al.* 2009, Verma *et al*. 2009, Regnault-Roger *et al.* 2012, Isman and Grieneisen 2014). The mode of action for some of these botanical essential oils (particularly for monoterpenoids) seems in part to involve neuroexcitation due to the inhibition of acetylcholinesterase (Grundy and Still 1985, López and Pascual-Villalobos 2010, Askar *et al.* 2016).

For termite control, the following characteristics of botanical essential oils might make them a promising option to be combined with a heat treatment approach. First, essential oil constituents are relatively volatile, and so may function as fumigants (Isman 2006, Koul *et al*. 2008, Ma *et al.* 2014). That is, even if the pest insect does not directly contact the oil, the insects may still be killed by its vapor. Secondly, many essential oil constituents exhibit some degree of repellency (Nerio *et al.* 2010, and references therein). Strong repellency would help to prevent the treated area from being occupied by the target pest. In fact, one essential oil constituent, *d*-limonene, has been tested as an insecticide for termite control. Fumigant action from vapor of *d*-limonene was effective in killing the Formosan subterranean termite (Raina et al. 2007). Workers exposed to the fumigant also exhibited reduced feeding activity, possibly due to the repellent activity of *d*-limonene, and they were unable to tunnel through more than ~ 3 cm of sand treated with 0.4% *d*-limonene before dying (Raina *et al*. 2007). For drywood termites, a registered insecticidal formulation of *d*-limonene is commercially available for localized injection treatment (Lewis and Rust 2009, Lewis and Forschler 2014). Based on a laboratory study, Rust and Venturina (2009) demonstrated that *d*-limonene has short-lasting but potent contact and fumigant toxicity for western drywood termites. When applied to wood, *d*-limonene also acted as a feeding deterrent for western drywood termites, even after the contact toxicity wore off (Rust and Venturina 2009). However, *d*-limonene was not considered for the current study due to its low flash point (50 °C, a typical target temperature for heat treatments).

The goal of the present study was to investigate whether the efficacy of whole structure heat treatment can be improved by incorporating insecticidal essential oil constituents (hereafter referred to as essential oils for simplicity) at locations that are shielded by a structural heat sink. The increased temperature during heat treatments will also facilitate volatilization of the essential oil, increasing its insecticidal action and repellency against western drywood termites. Two hypotheses are proposed: 1) that the presence of structural heat sinks would decrease the efficacy of heat treatments, and 2) that essential oils injected at the sites of heat sinks might help mitigate the effects of heat sinks, and allow control of drywood termite infestations with significantly shorter heating times and / or lower target temperatures compared to a traditional stand-alone heat treatment. To test the second hypothesis, a representative essential oil, methyl salicylate (hereafter referred to as MS), was first chosen based on its high fumigant toxicity (see Chapter II results), and relatively high flash point of 96 °C. Two separate experiments were conducted with MS: one in a laboratory setting and the other in a semi-field setting. The first study tested whether the addition of a MS will reduce the temperature required to get complete mortality of drywood termites. Small experimental arenas were constructed to simulate drywood termite galleries inside of pieces of wood. A gas chromatography (GC) oven was used to simulate various temperature conditions during heat treatments. The arenas containing termites were subjected to three different treatment conditions based upon the presence or absence of MS and the heat sink: (1) heat only, (2) heat + heat sink, and (3) heat + heat sink + MS.

The second study was conducted with larger arenas inside an experimental structure mimicking a typical house. A commercial heat treatment was conducted at the structure, and the termite control efficacy was compared among four different treatment conditions: (1) heat only, (2) heat + MS, (3) heat + heat sink, and (4) heat + heat sink + MS. If successful, the essential oils could be considered as "volatile adjuvants", making heat treatment a more effective and viable alternative to currently dominant whole structure fumigation methods for western drywood termite control.

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Chapter II. The use of an essential oil adjuvant to improve the efficacy of heat treatments targeting the western drywood termite: a laboratory study

Introduction

Heating infested wood 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). To address these limitations, different strategies might be considered to selectively treat these "hard-to-heat" areas of the structure to maximize the control efficacy of heat treatments.

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).

Using western drywood termites, the current 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, after comparing several essential oils, methyl salicylate (MS) was selected 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 were treated with a small amount of MS. Three different treatment strategies (i.e., heat only, heat + heat sink, and heat + heat sink + MS) were tested at several different temperatures to determine the relationship between temperature and termite mortality in each treatment.

The current 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 increase the minimum temperature needed 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 MS at the site of a heat sink might significantly lower the minimum temperature required to provide 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 lumber 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 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 a harborage. Each petri dish was provisioned with a piece of water-soaked wood (0.1 by 0.5 by 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 week prior to 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. Because 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 fumigant toxicity of the essential oil was of major importance. *D*-limonene (95% purity, XT-2000, Santee, CA), MS (99% purity, Millipore Sigma, St. Louis, MO), and eugenol (99% purity, Millipore Sigma, St. Louis, MO) were included in the experiment. One termite pseudergate was placed in a 20-ml scintillation vial (foamed polyethylene liner, DWK Life Sciences, Millville, NJ). A small section of balsa wood stick (0.3 by 0.6 by 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, Oak Brook, IL). After adding 10 µL of the essential oil to the cotton, the vial cap was screwed tightly on the vial. Controls were prepared following the same method, but the cotton ball did not receive any essential oil. 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 post-treatment. 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. Morbid 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 as 'mortality'.

Experimental Arena. To simulate termite galleries in infested structural lumber, experimental arenas were constructed from pieces of Douglas fir. The arenas (3.8 by 3.8 by 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 (0.2 by 0.7 by 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 by 3.8 by 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 prior to 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. 2.1).

A total of 10 pseudergates were added into each arena through the open end of the channel prior to 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 diam.) seated on a glass cylinder (6.5 cm diam. by 8 cm height, Fig. 2.2A). 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 + heat sink"). To simulate this condition, arenas were vertically placed on the surface of sand (600 g,

Fig. 2.1. One assembled arena (left) and disassembled component pieces.

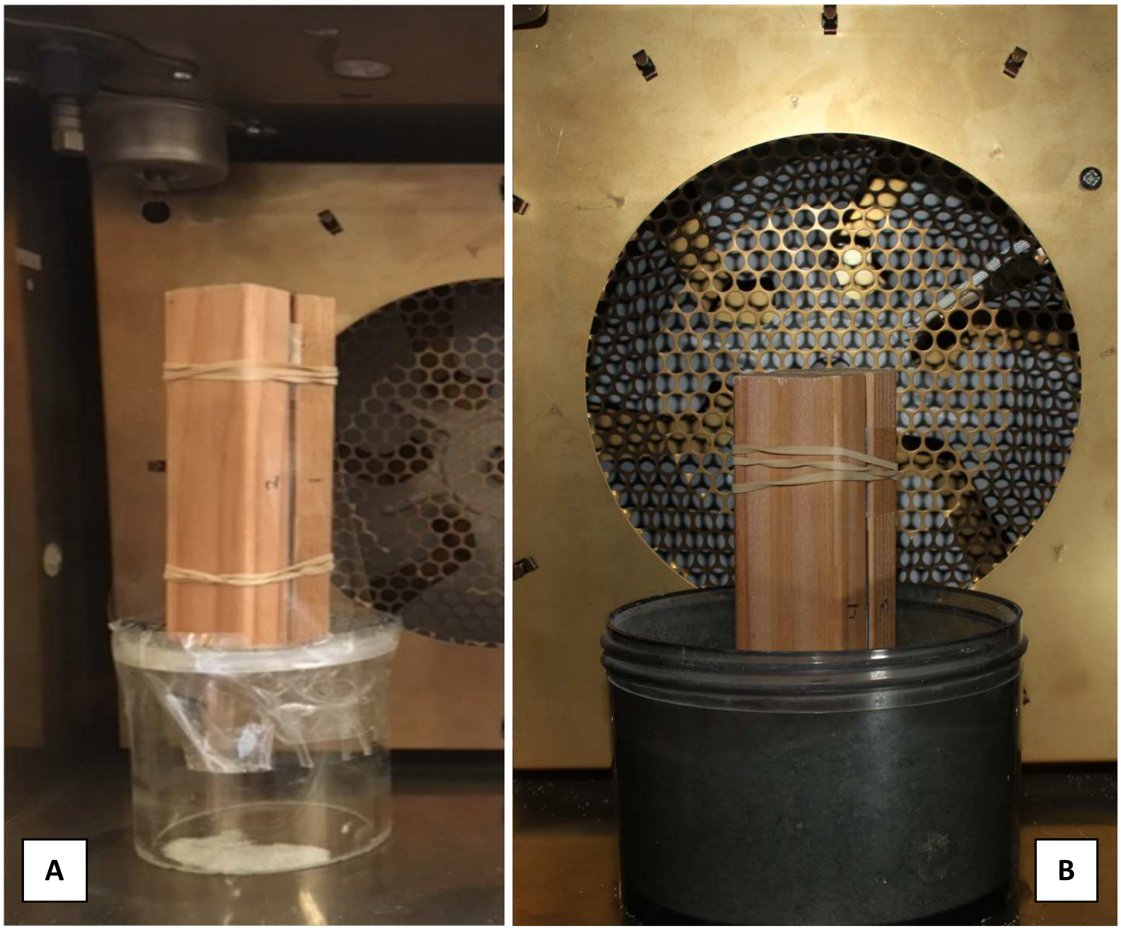

Fig. 2.2. Arena setups for each trial type. (A) An arena resting on wire mesh on top of a glass cylinder for heat only trials. (B) An arena seated in 600 g of sand for all trials utilizing a heat sink.

Quikrete, Atlanta, GA) contained in a plastic jar (11.2 cm diam by 7.2 cm height) (Fig. 2.2B). The end of the arena with the cotton plug was embedded in the sand, at a depth of ~ 2 cm. To determine if the incorporation of MS treatment at the site of heat sinks would improve heat treatment efficacy, 20 µL of MS were added to the cotton plug prior to placement in sand using a 10 - 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. 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 by 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 overall average 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, trials continued for 2 h once the GC oven reached the target temperature. 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 8 times.

Two arenas from a single treatment were treated at a time in the GC 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 (Fig. 2.3). The heat treatments were conducted for 2 h, starting at the time when the oven reached the set target temperature, which took about 5-10 sec. After each heat treatment, the oven was turned off and the arenas were removed. 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 diam by 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 morbid termites in

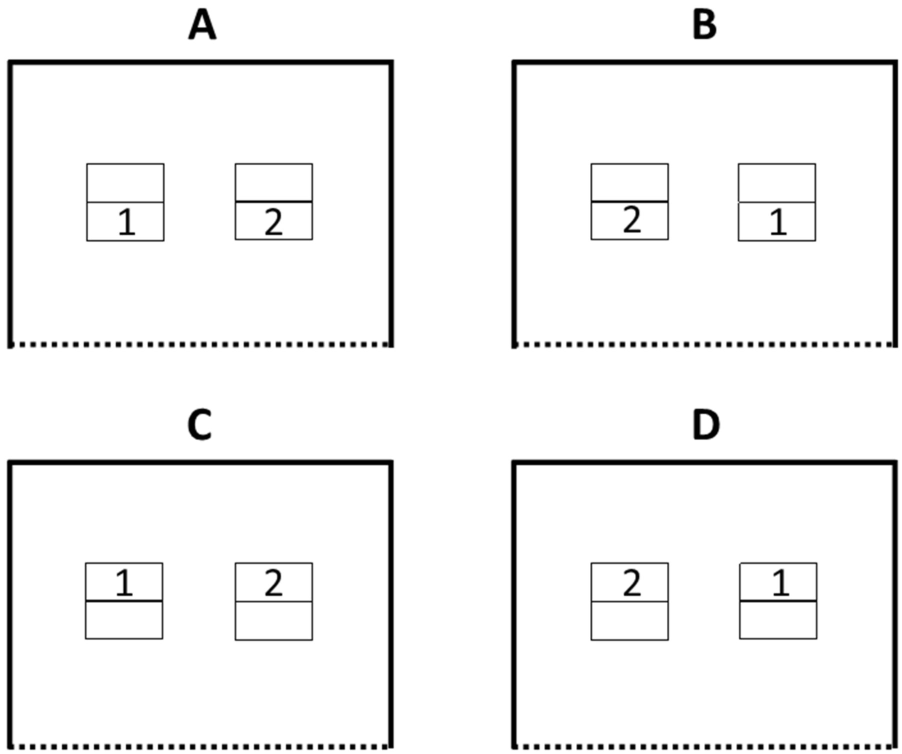

Fig. 2.3. Top-down diagram illustrating the four configurations used for trials; the dashed line represents the door of the oven. The arenas are numbered to illustrate directionality and placement within the oven for each configuration (i.e., arena "1" is placed on the left, facing forward, for configuration A).

each arena was counted immediately after the termites were transferred to the petri dish, and daily for the next 14 d (In this experiment, 'mortality' included both dead and morbid termites). Death was characterized by no response to light prodding with forceps. Morbidity was characterized by weakened / spastic leg movements and inability to grip the substrate. 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 timepoint (t). A log-rank test was used to determine if 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 Kisinski 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 8 replications (% mortality of 80 termites) were used for each combination of treatment and temperature. Based on the trend over time and background mortality evident in the control (i.e., exposed to ambient temperature) by 14 d, subsequent analyses requiring the choice of a single data set from a specific time point used the 7 d data (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 *et* al. 2018). 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 three 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). Three explanatory variables were included in the model: temperature, treatment (heat only, heat + heat sink, or heat + heat sink + MS), and colony of origin. 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). In order 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 (AIC). A global model was used including temperature, treatment, and colony of origin as explanatory variables, alongside a term to represent the interaction between temperature and treatment.

The resulting ‘best’ model was used to determine if 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. Secondly, 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 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 Figure 2.4. 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, while 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 post-treatment revealed that after 4 d of exposure the fumigant action of eugenol was sufficient to kill all termites, whereas *D*-limonene fell to a minimum of ~ 0.7 survivorship even after 5 d. Based on the results, MS was the only candidate to act as a satisfactory fumigant and was chosen for the subsequent experiments.

Heat Treatments. Overall cumulative mortality data for the heat treatment trials are summarized in Fig. 2.5. In "heat only" (Fig. 2.5A) and "heat + heat sink" (Fig. 2.5B),

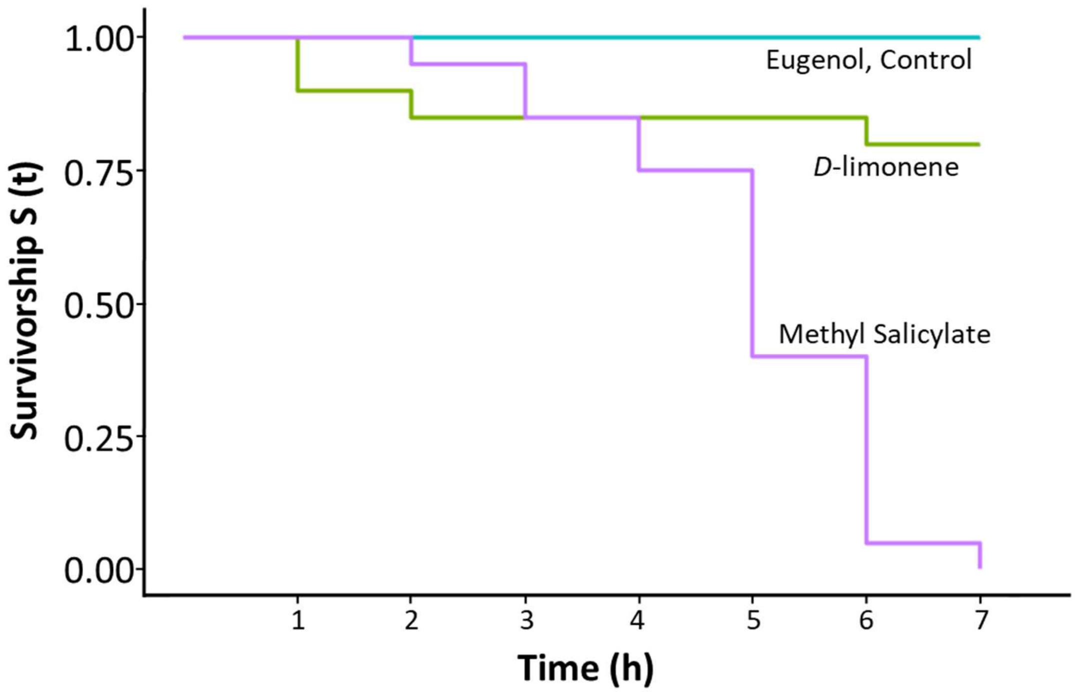

Fig. 2.4. Survival plots from fumigant toxicity tests. S(t) represents the estimated probability of an individual termite surviving past a given hour (1-7). Survival curves for eugenol and control (untreated) completely overlap.

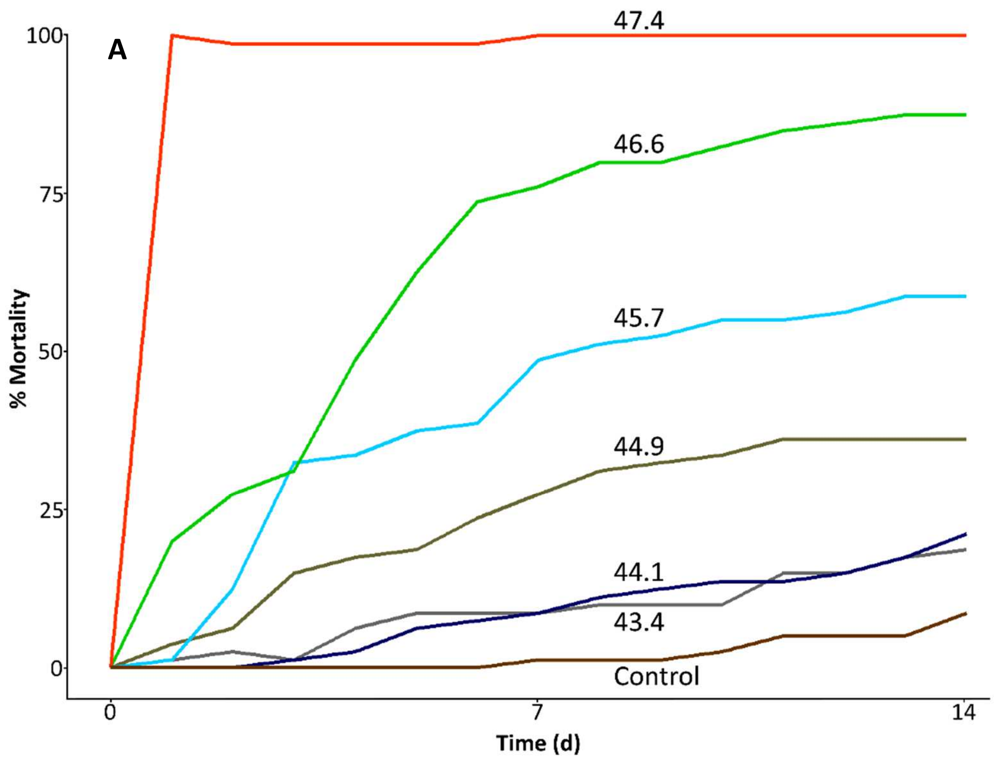

Fig. 2.5. Cumulative overall mortality for all temperatures (labelled next to lines in °C) within treatments, including the no-heat controls. (A) Mortality from heat only trials.

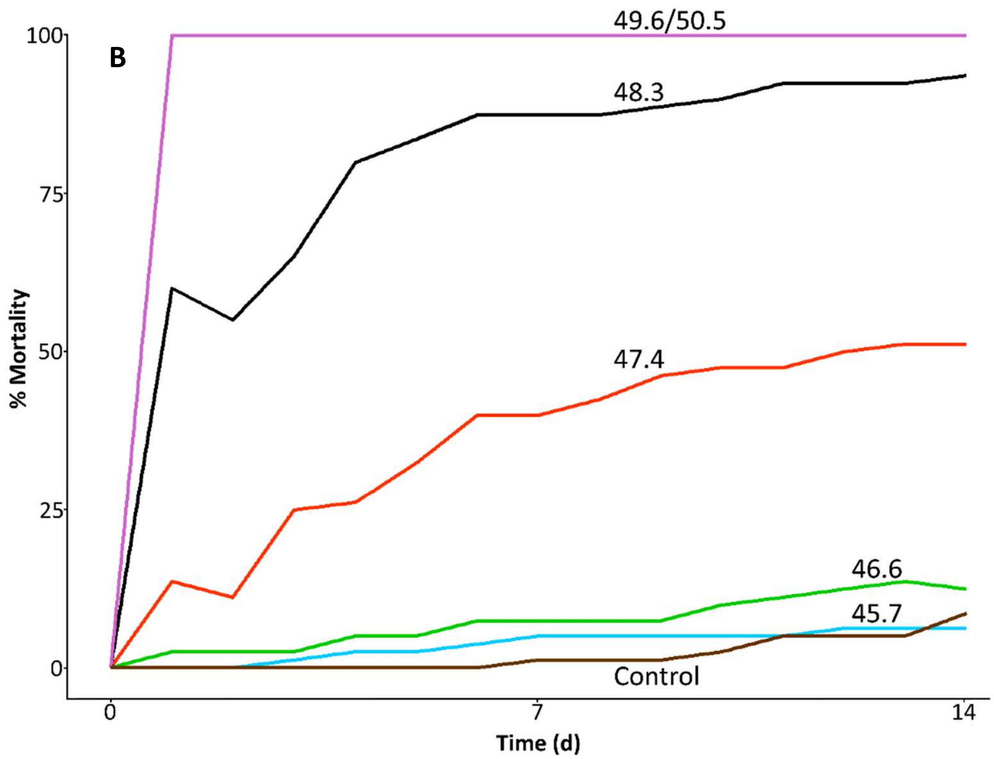

Fig. 2.5. Cumulative overall mortality for all temperatures (labelled next to lines in °C) within treatments, including the no-heat controls. (B) Mortality from heat + heat sink trials.

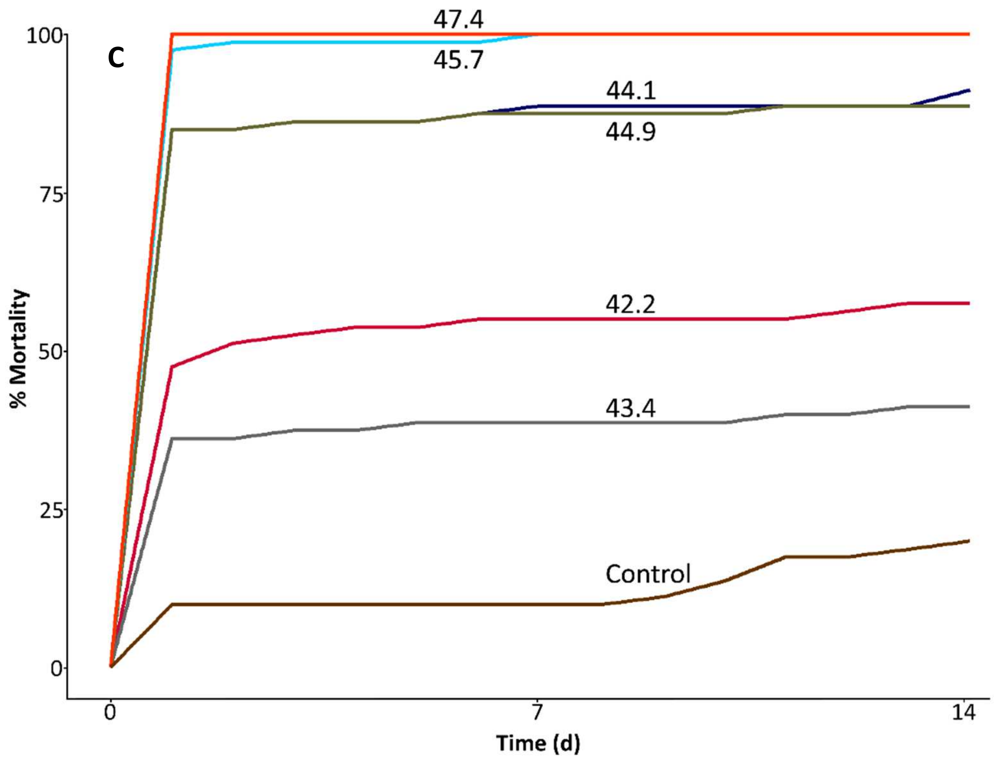

Fig. 2.5. Cumulative overall mortality for all temperatures (labelled next to lines in °C) within treatments, including the no-heat controls. (C) Mortality from heat + heat sink + MS trials.

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. 2.5C), 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). Additionally, 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.

Day 7 mortality data for all combinations of temperature and treatment are summarized in Table 2.1. 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”, and the highest range was used for “heat + heat sink”. For “heat only”, 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 had 10 or 1% mortality with or without MS, respectively.

Table 2.1. Western drywood termite mortality (± SEM) across all treatments and temperatures

Treatment	Temperature (°C)	n	mortality (%) at 7 d (mean ± SEM)
Heat only	43.3	8	9 ± 4
	44.1	8	9 ± 3
	44.9	8	27 ± 8
	45.7	8	49 ± 8
	46.6	8	76 ± 6
	47.4	8	100
Heat + heat sink	45.7	8	5 ± 2
	46.6	8	7 ± 2
	47.4	8	40 ± 8
	48.3	8	87 ± 5
	49.6	8	100
	50.5	8	100
Heat + heat sink + MS	42.2	8	55 ± 12
	43.3	8	39 ± 13
	44.1	8	89 ± 11
	44.9	8	88 ± 8
	45.7	8	100
	47.4	8	100
No heat control	28.3	8	1 ± 1
No heat control + MS	28.3	8	10 ± 5

Generalized Linear Models. $LTemp_{50}$s and $LTemp_{99}$s for three different treatments are provided in Table 2.2. 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 $_{99}$ values at 47.5 °C and 49.6 °C, respectively. However, the strongest difference was 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 (Fig. 2.6). The automated model selection was used to include temperature, treatment, and colony of origin as explanatory variables, alongside a term to represent the interaction between temperature and treatment. The effects plots and the output of the corresponding GLM, "heat only" had a significantly higher expected probability of mortality than "heat + heat sink" ($Z = -3.62$, $P < 0.001$) (Fig. 2.6A). Similarly, "heat + heat sink + MS" had a significantly higher expected probability of mortality than "heat + heat sink" ($Z = -6.30$, $P < 0.001$) (Fig. 2.6B).

Survival Analyses. Results for the Kaplan-Meier survival analysis for the heat treatments at 45.7 °C are shown in Figure 2.7. 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

Table 2.2. $LTemp_{50}$ and $LTemp_{99}$ values for each treatment with 95% confidence intervals

Treatment	LTemp50 (°C)[a]	LTemp99 (°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.

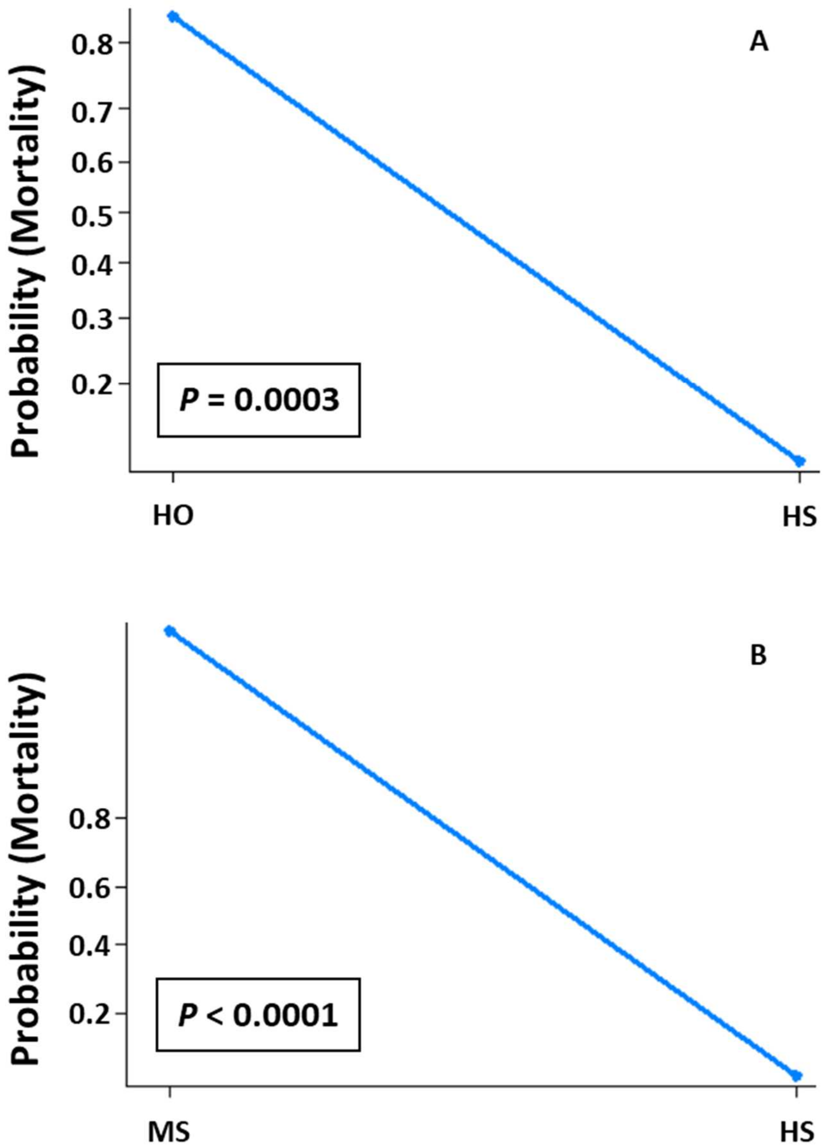

Fig. 2.6. Effects plots illustrating the differences in probability of mortality between treatments across all temperatures. Treatment abbreviations: “HO” = heat only, “HS” = heat + heat sink, “MS” = heat + heat sink + MS. (A) Mortality probabilities for “heat only” and “heat + heat sink” treatments. (B) Mortality probabilities for “heat + heat sink + MS” and “heat + heat sink” treatments.

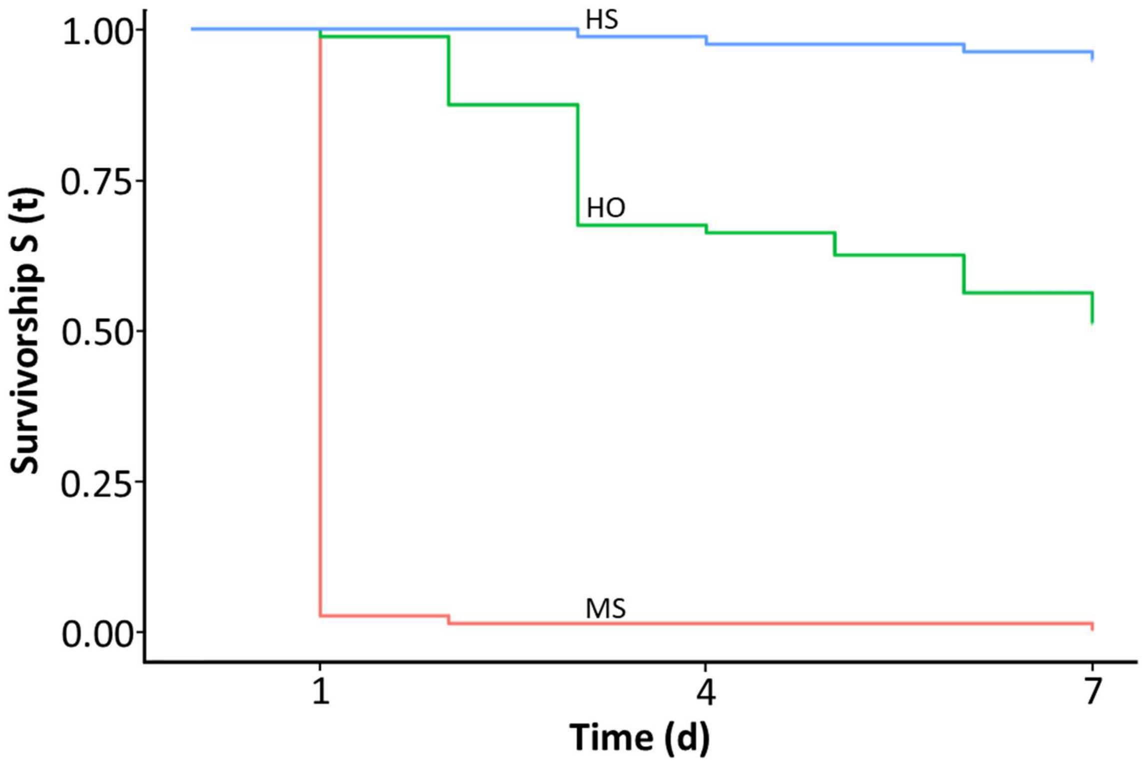

Fig. 2.7. Survivorship curves for each treatment type at 45.7 °C. Each interval represents the probability of an individual termite surviving until a given day (1-7) during the observation period. Treatment abbreviations: “HO” = heat only, “HS” = heat + heat sink, “MS” = heat + heat sink + MS.

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 + morbidity) 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. Clearly, MS is a superior fumigant to either eugenol or *D*-limonene and was selected for the study.

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. 2.5A and B). After 1 d post-treatment, morbidity 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. Based on the mortality trends in heat treatments and no heat controls, and the high proportion of overall mortality observed by day 7, mortality data up to day 7 post-treatment were chosen for subsequent statistical 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 is used. If the target temperature could be lowered, the heat treatment would take less time to complete, potentially reducing the cost and inconvenience that the occupants experience (i.e., duration of evacuation). 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 objects or structural features during 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. Presence of heat sinks in the structure might make heat treatments more difficult, necessitating either higher temperatures and/or longer heating duration to achieve lethal conditions in the “hard-to-heat” areas. Similarly, the effects plot analysis indicated that the “heat + heat sink + MS” treatment had significantly higher mortality probability than did the “heat + heat sink” treatment. This supports the hypothesis that 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. 2.7). 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. 2.5A 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. 2.5C). This difference indicates that 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 sub-lethal temperature conditions simultaneously? There are at least two 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 physical contact with the essential oil quickly kills the termites. Alternatively, 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 sub-optimal in providing quick mortality when used separately) would quickly kill the termites. The insecticidal action of essential oils also might increase with temperature. Future research on termite behavior (i.e., location and various parameters in locomotion) during heat treatment is 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, this study took place under controlled laboratory conditions. The wooden arenas were likely to have been heated fairly evenly compared to 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). Additionally, 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 to what was simulated in the current experiment. This discrepancy might influence the interpretation of the current laboratory results, with less 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. However, if the temperature inside an infested piece of wood never approaches the target temperature due to a heat sink, some termites may survive the treatment. Thus, the importance of overcoming the impact of structural heat sinks still remains.

The current study had two hypotheses: first, that the presence of a structural heat sink might result in significantly increased temperature needed for complete mortality, and second, that 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 current 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. Additionally, 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 locations shielded by heat treatments) 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 current study. To demonstrate the benefit and practicality of the combined use of botanical essential oils and heat treatment for drywood termites, a more realistic simulation needs to be conducted using a larger structure and actual heating protocols that are currently used in typical heat treatments.

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Chapter III. The use of an essential oil adjuvant to improve the efficacy of heat treatments targeting the western drywood termite: a semi-field study

Introduction

Results from the laboratory trials demonstrated that the presence of structural "heat sinks" could pose a significant challenge in killing all termites present in a piece of wood. However, the addition of methyl salicylate (MS, a major chemical constituent of wintergreen oil) at the sites of "heat sinks" prior to the heat treatment effectively addressed this challenge, possibly by preventing termites from taking refuge in these locations or by directly causing mortality (see Ch. II). These findings support the concept that localized injection of insecticidal essential oils at the sites of heat sinks can be incorporated in a treatment protocol to improve the efficacy of heat treatments for the western drywood termite. However, due to the limited scale of the laboratory trials (i.e., small wooden experimental arenas, heat treatment inside of a gas chromatograph oven), generalized conclusions regarding the efficacy of the proposed approach require further tests in more realistic scales.

Whole-structure heat treatment for western drywood termite control has been previously simulated 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. The structure was heated with several propane heaters until the thermocouples showed a target temperature of 50 °C and was maintained for 1 h. After this treatment, 100% mortality was achieved in all tested boards except for boards that were positioned against a concrete foundation wall in the subarea of the structure (8.9% survival), suggesting that the concrete foundation may have functioned as a structural heat sink and shielded some termites from being exposed to lethal temperatures.

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. To simulate the heat treatment on a more realistic scale, the following modifications were made to protocols used for the laboratory trials (Ch. II). First, larger wooden arenas than those used in laboratory trials were constructed from structural lumber to simulate infested wall studs or other structural boards. The arenas 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 arenas within each zone. Second, heat treatment of the experimental structure was conducted by licensed pest management professionals (PMPs), following the protocols that would be used in a typical heat treatment targeting drywood termites.

This semi-field 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. Wood was first checked for termites using a microwave termite detection device (T3i All Sensor, Termatrac, Ormeau, QLD), then cut into small pieces to reveal galleries within, and finally the termites were collected using soft-touch forceps and a camel-hair brush. Termite were sorted by colony and collection date and placed into petri dishes for observation. Each dish was lined with a filter paper disc (90 mm diam, Millipore Sigma, Darmstadt, Germany), and the termites were provided with balsa wood pieces for food and harborage. Once per week, a piece of water-soaked wood (0.1 by 0.5 by 1.5 cm) was placed in each petri dish to provide moisture. All termites were observed for at least 1 week prior to use in the experiment, and they were used within 60 d of collection. Dead termites were removed from each dish once per week to ensure that only healthy individuals were used.

Experimental Arena. To simulate infested structural lumber, experimental arenas were constructed from Douglas fir. The arenas (5.08 by 10.2 by 25.4 cm) were designed to house a group of western drywood termites during whole-structure heat treatments. Each arena was composed of two pieces of wood (2.54 cm height each). To hold the termites, a narrow channel (0.7 by 1.35 by 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 by 10.2 by 25.4 cm, Plaskolite, Columbus, OH) was placed over the top of the channel-bearing bottom piece of the arena to facilitate observation of the termites. The acrylic sheets were rinsed with 2% Liquinox detergent (Alconox, White Plains, NY) and deionized water prior to placement in an arena. The top piece was placed on the bottom piece, with the acrylic sheet pressed between them, to form a complete arena (Fig. 3.1).

Termites were added to the experimental arenas prior to the heat treatment trials. A total of 20 pseudergates were added directly into the channel of each arena. Of these 20, 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, the two pieces of wood were placed together and secured using two heat resistant cable ties (Xtreme Ties, Gardner Bender, Menomonee Falls, WI).

In addition to the experimental arenas containing termites, six arenas were prepared for the purpose of monitoring the temperature inside the arenas during the heat treatment. These temperature monitoring arenas 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. 3.1. Disassembled arena showing the top (right) and bottom (left) pieces of arena. A sheet of acrylic, the cotton balls, and termites inside the channel are shown on the bottom piece of the arena (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. 3.2). This cubic building (6.1 by 6.1 m floor, with an area of 37.2 m^2 and a volume of 154 m^3) had been 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. To simulate infested studs in the walls of a structure, the experimental arenas were placed in the aboveground wall voids (Fig. 3.3A). Of the four walls in the structure, only the west, north, and east walls were used for trials due to structural limitations in the south side. To access the wall voids, two sections of drywall touching the corners and extending approximately 0.9 m inwards were cut open per side. To allow placement of arenas 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 arenas were placed at the heights of 80, 130, and 180 cm from the floor. Two long metal screws partially screwed into the wall at each height served as a bracket on which the arena was placed. The brackets were insulated with a folded piece of paper towel and a tape. One temperature monitoring arena was placed at a height of 25 cm in the left wall void of each wall. In total, eighteen experimental arenas were placed in the wall voids.

Fig. 3.2. "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. 3.3. Two different locations where the experimental arenas were placed. (A) Wall void. Brackets were used to place multiple arenas within a single wall void space. Four arenas are shown in the photo, and the bottommost one is a temperature monitoring arena. (B) Subarea. The arenas were placed on the top of concrete perimeter wall. The leftmost arena is a temperature monitoring arena.

To simulate an infested sill plate, the experimental arenas were placed on the top edge of the concrete perimeter walls in the subarea (Fig. 3.3B). 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 arenas and the perimeter wall surfaces, 250 g of sand was first placed on each location, and the arena 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 arenas and one temperature monitoring arena (leftmost location) were placed on each perimeter wall. See Fig. 3.4 for the approximate location of each arena in the test structure.

For both wall void and perimeter wall locations, half of the arenas were treated with methyl salicylate (MS) (see Methyl Salicylate Treatment). Additionally, some arenas 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 arenas were tested.

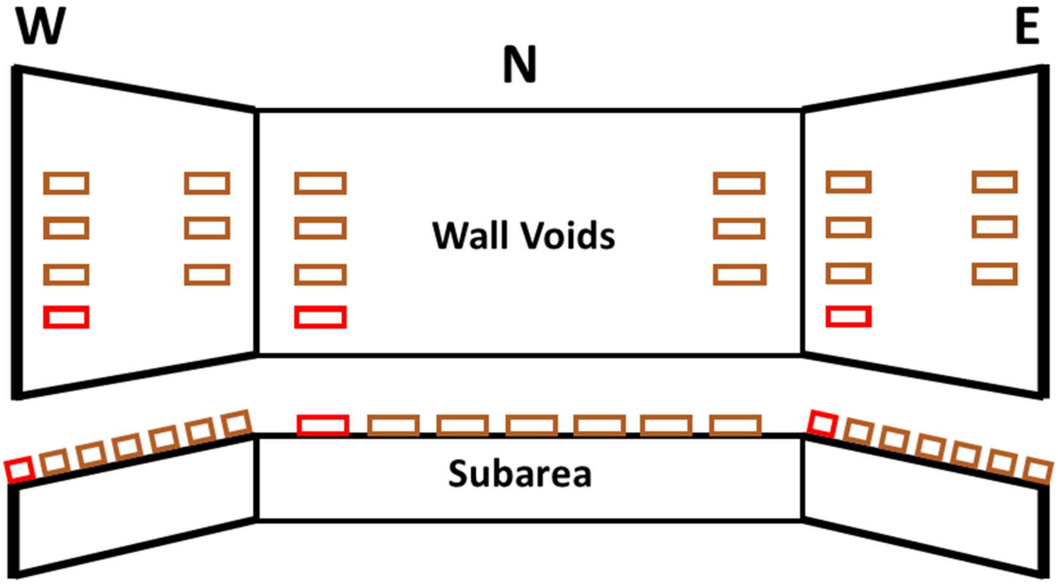

Fig. 3.4. A schematic diagram showing the relative positions of the arenas (total 42 arenas are shown) in the wall voids and subareas of the west, north, and south walls. Arenas outlined in red denote temperature monitoring arenas. Not drawn to scale.

Methyl Salicylate Treatment. For the arenas that were assigned to MS treatment, 80 μL of methyl salicylate (99%, Sigma-Aldrich, St. Louis, MO) was added to each of two cotton balls in the arena (160 μL per arena) 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 arena channels from the small-scale trials (see ch. II) and the current study. The application of MS was made immediately prior to placing the arenas in the test structure. To minimize cross contamination with MS volatiles between different arenas / treatments, particularly in the subarea where arenas were placed in close proximity, the gap between the two pieces of the arena was sealed by wrapping the side of the arena (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 (Fig. 3.5), 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, Anaheim, CA) were placed in the main space of the Villa Termiti to provide air flow and reduce the incidence of heat stratification.

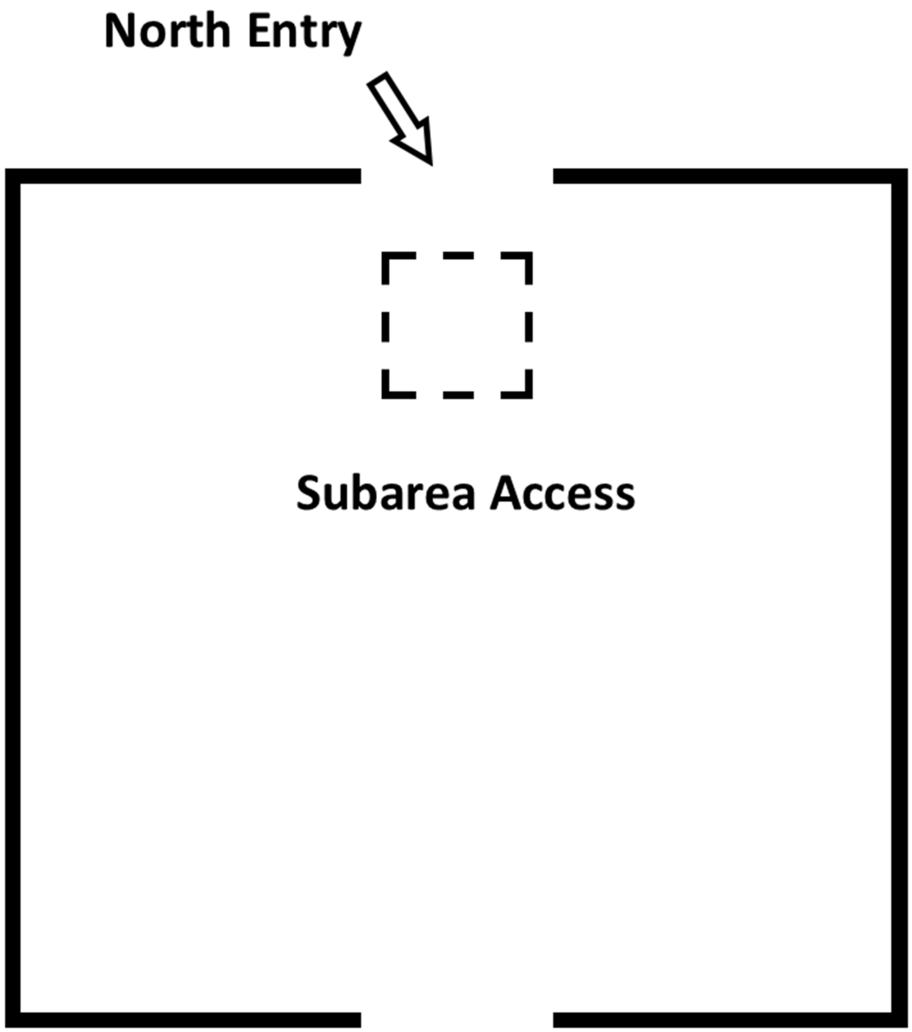

Fig. 3.5. A schematic diagram showing the location and approximate size of the hatch leading from the main room of the test structure into the subarea. Not drawn to scale.

According to Forbes and Ebeling (1987), exposure to 48.9°C for 30 min is sufficient to kill drywood termites. Based on this, a target temperature of 49°C was chosen for both treatments. The temperature measured within each temperature monitoring arena 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 observational arenas 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 the standard heat treatment protocol. However, 30 min prior to the end of the trial, temperatures recorded within the subarea arenas 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 sub-optimal, short protocol for heat treatment. In both cases, the structure was opened and allowed to cool for 10 min before the arenas were retrieved.

For trial 1, 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 arena 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 arenas were brought back to the laboratory, they were placed on a counter in separate piles for wall void, subarea, and no heat control arenas to minimize any cross contamination. Each arena was then opened, and the top and bottom of the arena were placed interior side up on the counter. Forceps were used to transfer the termites from each arena to a plastic petri dish (60 mm by 15 mm, Corning Inc., Corning, NY) for observation. Each petri dish was provisioned with a small piece of paper towel to serve as substrate and a food source. Immediately after the conclusion of treatment and once a day for 14 d afterwards, the number of dead or morbid termites was counted for each arena based on the behavioral response of termites when probed with forceps. For the purposes of the current experiment, 'mortality' included both dead and morbid termites. Death was characterized by no response to light prodding with forceps. Morbidity was characterized by weakened or spastic leg movements and inability to grip onto the substrate. Separate pairs of forceps were used to handle termites from the treatments with or without MS to prevent 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 arenas themselves, data from the temperature monitoring arenas 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 arenas were placed in each area of the structure (1 for each side), the average recorded temperatures at each timepoint were used.

For each treatment, overall cumulative mortality data (pooled from 9 replications) were graphed to illustrate trends in mortality over time (Wickham 2016). The pooled data from 9 replications (% mortality of 180 termites) were used for each treatment. Based on the mortality trends observed in the treatments and no heat controls, subsequent analyses used the data from days 1-7 (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 as well as the no-heat controls (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 the wall void and subarea locations and to determine whether % mortality significantly differed between 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).

Survivorship data between day 1 and day 7 post-treatment 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 test with BH-adjusted *P* values (Kassambara and Kisinski 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 arenas, are summarized in Fig. 3.6. 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 arenas closely matched the ambient wall void temperature across the trial duration. In contrast, the ambient temperature in the subarea remained higher than the average temperature within subarea arenas, 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 arenas (11.7 °C) was recorded at the start of the trial. The greatest difference in temperature between the interior and exterior of the subarea arenas (22.9 °C) was recorded 8 min prior to turning off the heater that blew directly into the subarea.

Termite Mortality. Overall cumulative mortality data from both trials and the no heat controls are summarized in Fig. 3.7. Although a substantial portion of the mortality was accounted for by day 1, some of the surviving termites were affected by the treatment, and mortality increased in the subsequent observations. Cumulative mortality for all treatments and controls by day 7 accounted for 97% of the total mortality (day 14 mortality). For these reasons, the cumulative mortality data up to day 7 post-treatment were used for the subsequent statistical analyses.

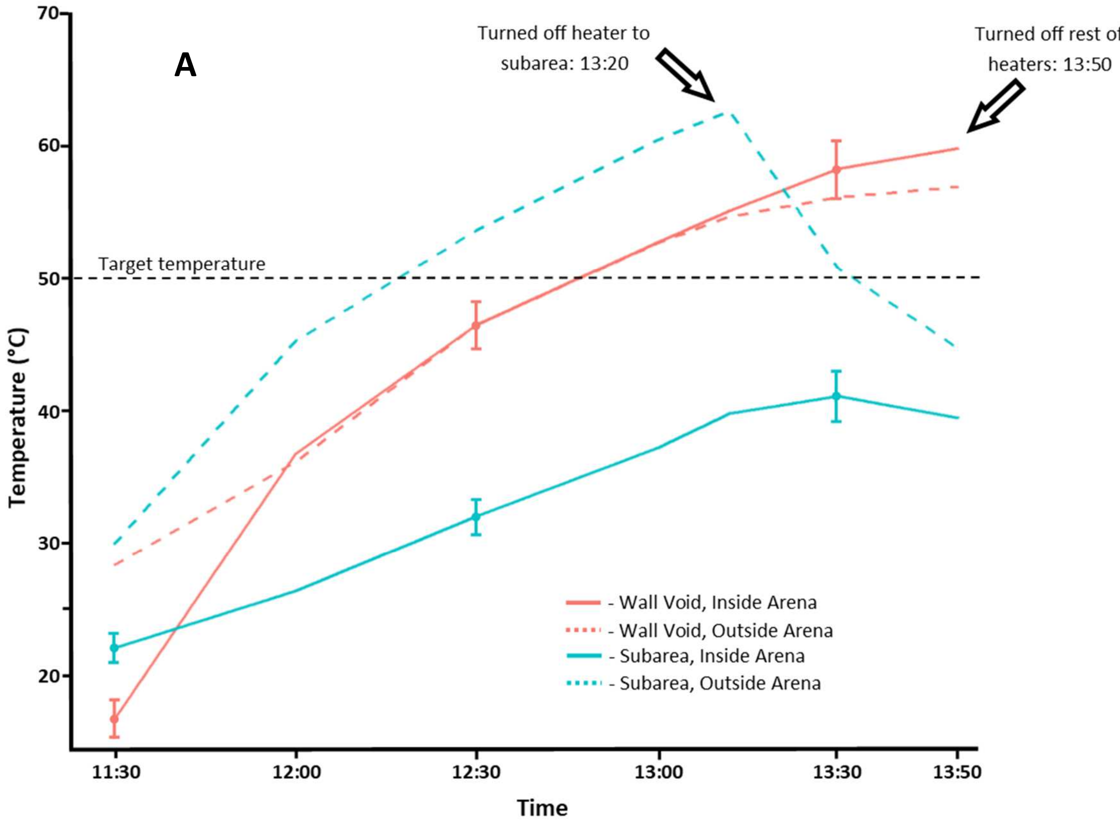

Fig. 3.6. Temperature measurements both within temperature monitoring arenas using thermocouples, and outside arenas using data loggers. Lines for temperature monitoring arenas represent the average temperatures from three arenas, and the SEM is indicated at 0, 1, and 2 h after beginning treatment. (A) Trial 1. Data from inside and outside arenas are shown.

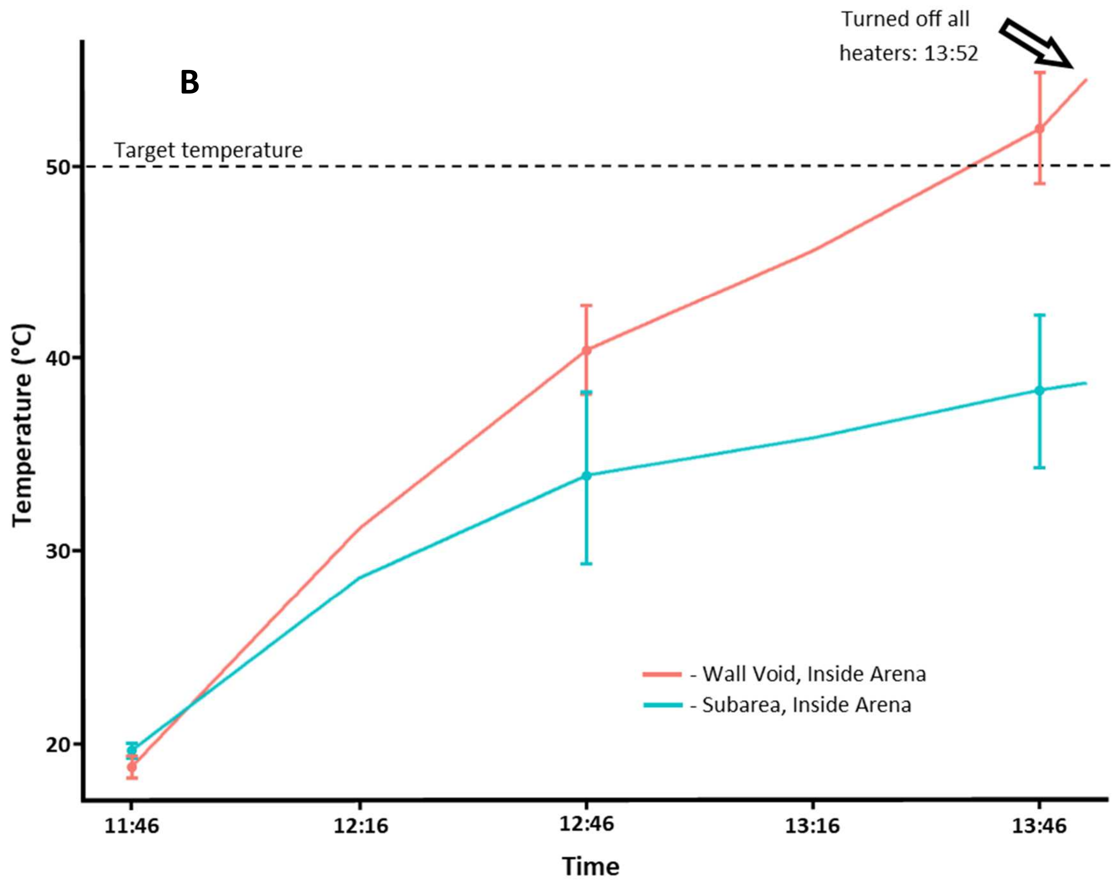

Fig. 3.6. Temperature measurements both within temperature monitoring arenas using thermocouples, and outside arenas using data loggers. Lines for temperature monitoring arenas represent the average temperatures from three arenas, and the SEM is indicated at 0, 1, and 2 h after beginning treatment. (B) Trial 2. Only data from inside arenas are shown.

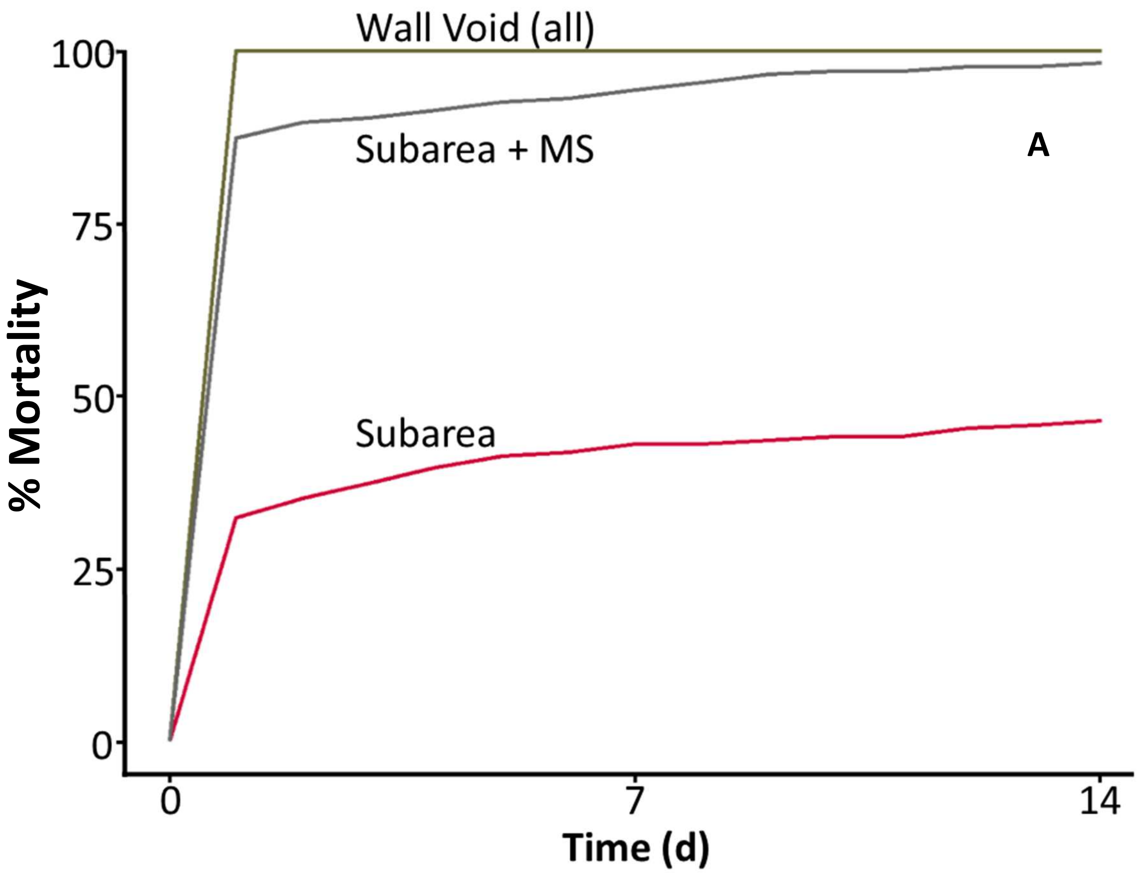

Fig. 3.7. Overall cumulative % mortality from pooled data (of 180 termites for each treatment or control). (A) Mortality from heat treatment trial 1. Mortality lines from “wall void” and “wall void + MS” overlap.

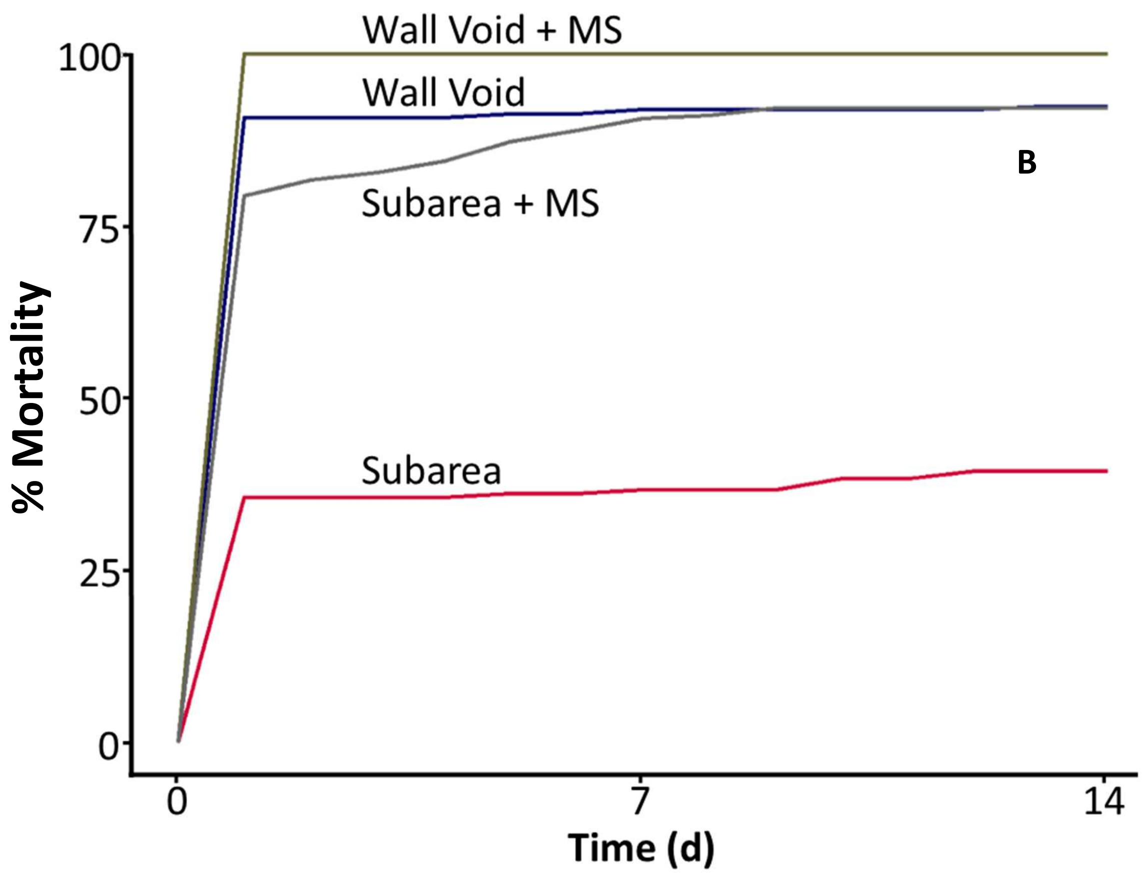

Fig. 3.7. Overall cumulative % mortality from pooled data (of 180 termites for each treatment or control). (B) Mortality from heat treatment trial 2.

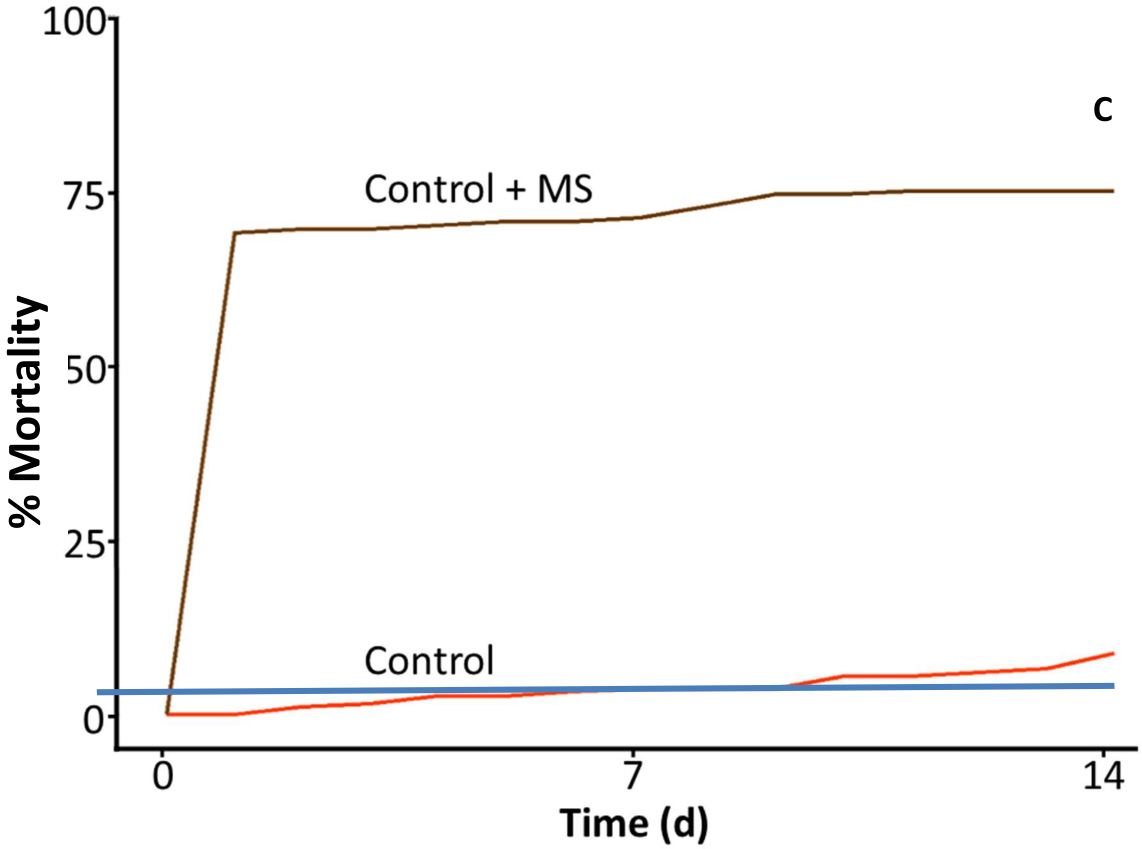

Fig. 3.7. Overall cumulative % mortality from pooled data (of 180 termites for each treatment or control). (C) Mortality from no heat controls.

Day 7 mortality data for both trials as well as the controls are summarized in Table 3.1. Regardless of the presence or absence of MS, all treatments in wall voids achieved 92-100% mortality. In fact, all wall void treatments had 100% mortality except the "no MS" treatment, in which one arena had 70% survival. 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 36 and 44% 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.

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, $P = 0.0035$) and the no-heat controls with and without MS ($H = 8.99$, df = 1, $P = 0.0027$). 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 that "heat / subarea / no MS" was significantly less effective in killing termites than either wall void treatment ($\alpha = 0.05$), with no other significant differences between treatments detected. Additionally, a Wilcoxon rank sum test indicated that "no heat / with MS" killed more termites than "no heat / no MS" ($P = 0.0031$).

Table 3.1. Western drywood termite mortality across all treatment conditions and trials at the test structure

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

[a]Values for % mortality represent the percentage of 20 individual pseudergates ($n = 9$)

Survival Analyses. Survival curves for the heat treatment trials and no-heat controls are provided in Fig. 3.8. Survivorship in “heat / wall void / no MS” quickly dropped to 0 in trial 1 (Fig. 3.8A) and to a very low level (~ 0.1) in trial 2 (Fig. 3.8B), with “heat / subarea / no MS having a much higher survivorship (~ 0.6 – 0.65) in both cases. Additionally, “heat / subarea / with MS” survivorship steeply declined to low levels (~ 0.15-0.2) and remained far below “heat / subarea / no MS” (trial 1: Fig. 3.8C, trial 2: Fig. 3.8D). 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, $P < 0.001$). Pairwise comparisons of the survivorship curves revealed that termites in “heat / wall void / no MS” had significantly lower odds of survival than did termites in “heat / subarea / no MS” for both trial 1 and 2 (log-rank test: $P < 0.001$ for each trial), providing further evidence that the presence of the heat sink in the subarea increases survivorship. Additionally, there was evidence that “heat / subarea / no MS” had a significantly lower impact on survivorship than “heat / subarea / with MS” in trial 1 and trial 2 (log-rank test: $P < 0.001$ for each trial), showing that treatment with MS increases mortality despite the presence of a heat sink. Finally, it was found that “no heat / MS” resulted in significantly lower survivorship than “no heat / no MS” (Fig. 3.8E, log-rank test: $P < 0.001$), indicating that even in the absence of a heat treatment, fumigant and/or contact action from MS can cause substantial mortality.

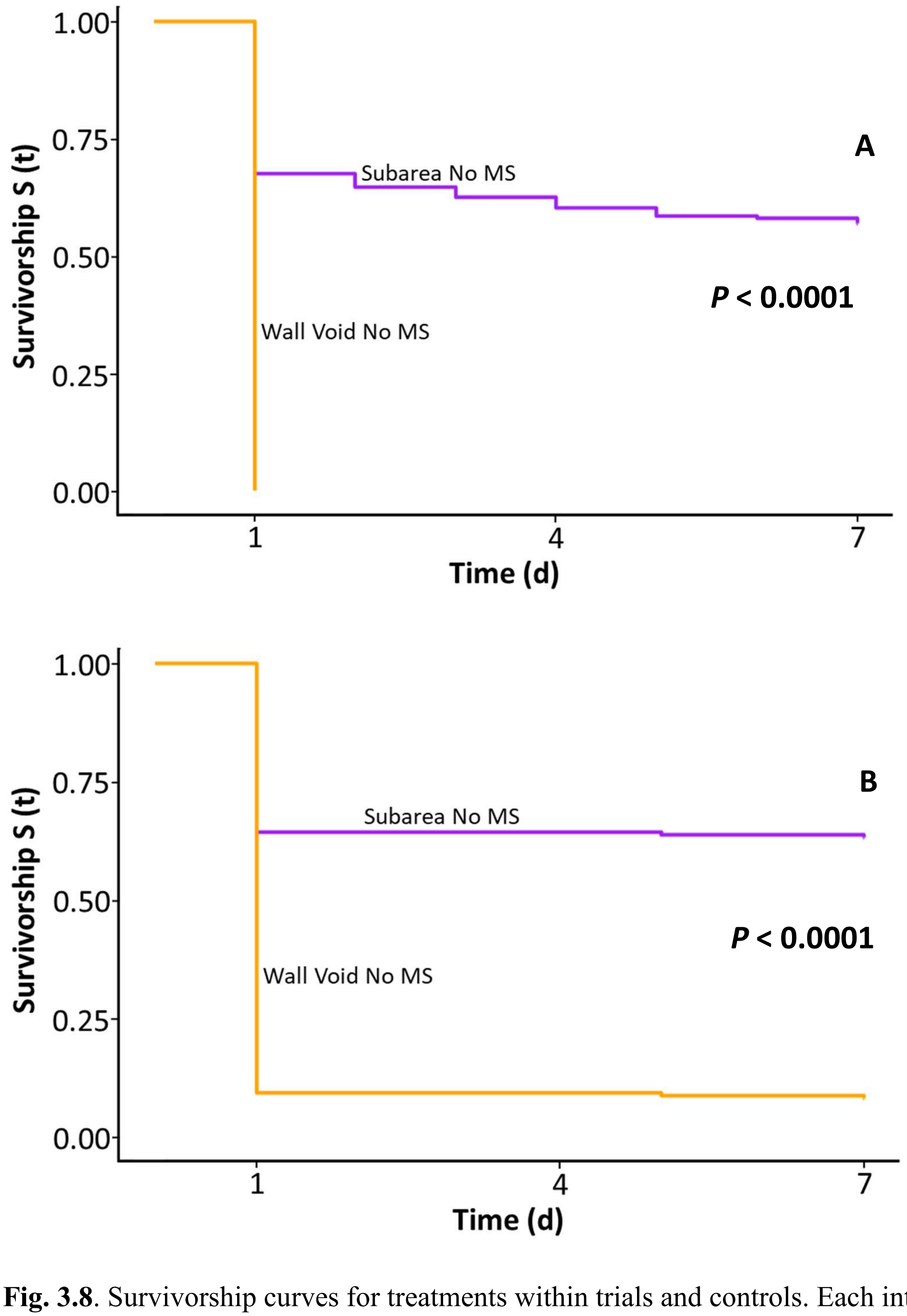

Fig. 3.8. Survivorship curves for treatments within trials and controls. Each interval represents the probability of an individual termite surviving until a given day (1-7) during the observation period. (A) Wall void and subarea treatments (trial 1), both without MS. (B) Wall void and subarea treatments (trial 2), both without MS.

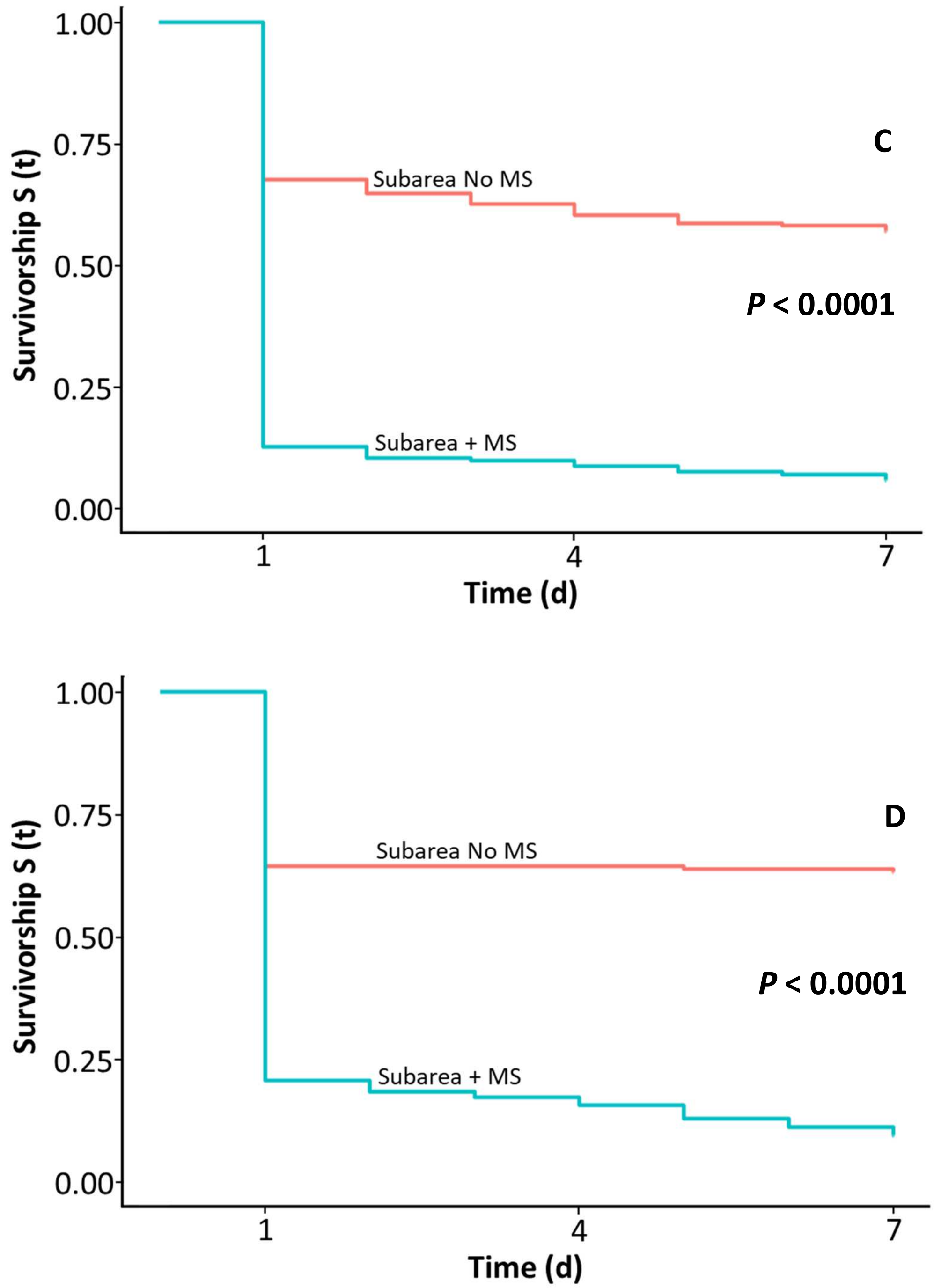

Fig. 3.8. Survivorship curves for treatments within trials and controls. Each interval represents the probability of an individual termite surviving until a given day (1-7) during the observation period. (C) Subarea treatments (trial 1), with and without MS. (D) Subarea treatments (trial 2), with and without MS.

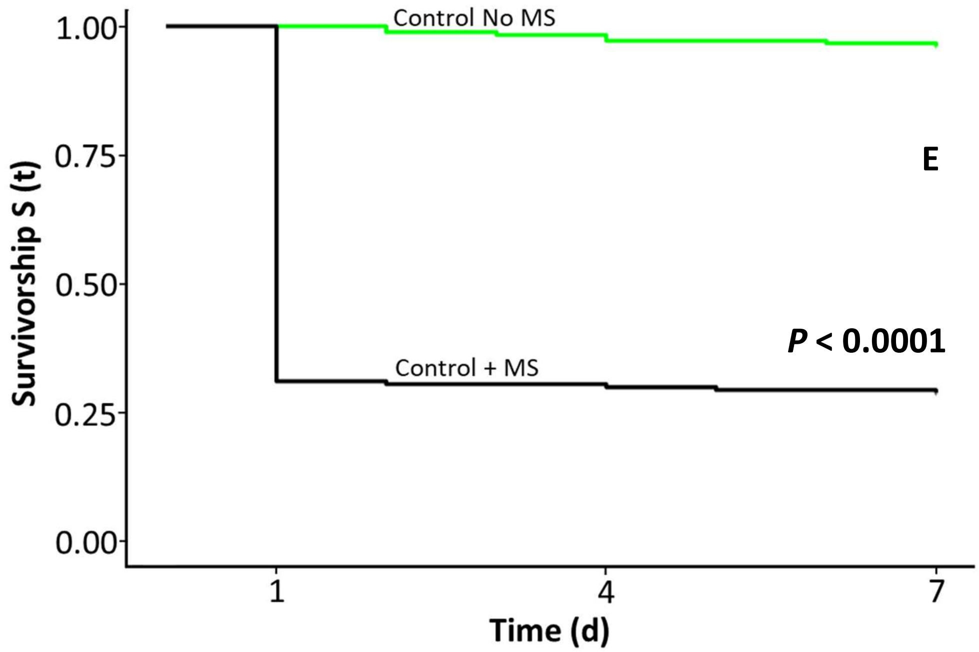

Fig. 3.8. Survivorship curves for treatments within trials and controls. Each interval represents the probability of an individual termite surviving until a given day (1-7) during the observation period. (E) No heat controls, with and without MS.

Discussion

Overall cumulative mortality data over time indicated that mortality observed on day 1 post-treatment was not always indicative of final mortality. For example, in some treatments of the subarea (with or without MS), mortality continued to accrue after day 1 post-treatment. This may have been due to residual effects of exposure to heat, especially considering that the peak temperature in the subarea was higher than in the wall voids. Based on the mortality trends in heat treatments and no heat controls, mortality data up to day 7 post-treatment were chosen for subsequent statistical analyses.

Arenas 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 in the wall void treatments were predicted to experience high mortality after the heat treatment. In contrast, the presence of the heat sink (i.e., concrete perimeter wall) was predicted to significantly reduce the efficacy of heat treatment in the subarea. The observed temperatures in each area supported these expectations. In particular, the lower temperatures recorded within the subarea arenas when compared to the surrounding air demonstrates that there was a "heat sink" effect in the subarea. Additionally, the observed differences in day 7 mortality between wall void and subarea treatments (without MS) were consistent with the predictions. Mortality was drastically 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. However, the addition of MS to arenas 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 the overall 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. First, this might be due to the fact that only 160 μL of methyl salicylate were added per arena, which would likely be less than in a real structural treatment. Secondly, survival mostly occurred in one arena from trial 1 (42.9% mortality), and two arenas from trial 2 (68.4 and 50% mortality). Average mortality among the remaining arenas 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 arenas. Thirdly, 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 arenas 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 termites as precisely as in this study. Finally, it should also be noted that the wooden arenas 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. It is likely that the wood in a structural heat treatment may remain at higher temperatures for longer compared to what was simulated in the current experiment, resulting in a more conservative estimate of termite mortality.

Compared to 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 less than 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.

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). However, 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. 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 lies in the long-lasting 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. 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 even with its relatively low scent profile (Shimomura *et al*. 2018). Based on our preliminary test, (-)-isopulegol volatiles resulted in 100%

mortality of 5 western drywood termites within 4 h. The use of less odoriferous essential oils warrants further investigation.

Based on the semi-field experiments, the current study showed that termites treated with heat in the presence of a structural heat sink experienced significantly less mortality than those without a heat sink present. Also, adding an essential oil constituent (methyl salicylate) in the termite gallery increased termite mortality, despite the presence of heat sinks, corroborating the findings of previous laboratory experiments. A combination of whole-structure heat treatment with localized injections of insecticidal essential oil constituents may not be feasible for all control scenarios, because directly accessing infested wood can be difficult or impossible. 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.

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Chapter IV. Conclusions

Structural heat treatment has a unique niche in drywood termite control – it is a non-chemical method that can address relatively large-scale infestations with no toxicity or residues (Forbes and Ebeling 1987, Eisenbrandt and Hotchkiss 2010). However, some areas in a structure may not become heated as quickly as desired due to the presence of structural heat sinks and other types of insulation (Lewis and Haverty 1996, Rust and Reierson 1998, Lewis and Forschler 2014). Some termites might be able to migrate to cooler areas of the infested wood as a response to elevated temperature (Cabrera and Rust 1996, Rust and Reierson 1998, Cabrera and Rust 2000), consequently increasing the possibility of colony survival and recovery after the heat treatment. To overcome this drawback, a combination method which incorporated targeted injections of insecticidal oil at the sites of heat sinks prior to the heat treatment was proposed. This study attempted to provide a proof of concept to verify that the concept has the potential for real-world application.

The current study provides strong support for the concept that localized application of insecticidal essential oil constituents at the sites of heat sinks would effectively reduce the likelihood of termites surviving heat treatment. In small-scale laboratory trials, termites exposed to methyl salicylate in the presence of a heat sink were killed at significantly lower temperatures than those that were only treated with heat. Trials in the larger-scale structure also showed that addition of the MS in the arena significantly improved the overall control efficacy (> 90 % mortality) even when heat sinks were present. Additionally, after the time necessary to bring the wall void arenas to the target temperature (75-90 min), arenas in the subarea remained ~15 °C below. The relatively high mortality observed when MS was added shows that control is possible without bringing all wood in the structure to the same target temperature.

In the semi-field experiment, some termites survived the heat sink treatments even with MS application (7 - 10% cumulative survivorship at day 7 post treatment). However, it is important to point out that the experiment employed a relatively small amount of oil added to each arena (160 μL). In a real application, it would be necessary to use sufficient amounts of material to ensure thorough coverage. One manufacturer of a termiticidal formulation of *D*-limonene recommends adding the *D*-limonene to each drilled hole for up to one minute (XT-2000 2017). Depending on the specific sprayer and pressure setting used by the applicator, it is likely that more essential oil would be added using this protocol than in the current study. As long as adequate coverage is achieved by adding enough oil, the refuges provided by heat sinks could be effectively eliminated. Furthermore, in the current study, the experimental arenas with termites were immediately removed from the heated structure, and the termites were also moved to clean containers for observation. However, in a real application, the termites inside of the infested wood will continue to be exposed to the heat and essential oil treatment for longer periods of time. This would likely result in higher mortality over time among individuals not killed immediately by the treatment, because even in the no-heat controls, termites that were exposed to MS for the treatment duration (~ 3 hours of exposure) experienced high mortality (72% at day 7 post-treatment).

Many essential oils and their constituents have topical toxicity against insects (Isman 2000; 2006, Cloyd *et al*. 2009). Due to the relatively high volatility of methyl salicylate and its demonstrated ability to kill drywood termites by vapor action (see Ch. II results), it is likely that it also acts as a fumigant once introduced into termite galleries (Shaaya *et al.* 1991, Jayasekara *et al*. 2005, Isman 2006, Phillips and Appel 2010). The fumigant action of MS would be further enhanced by the high temperature conditions during heat treatment. However, the current study did not determine the exact mechanism for termite mortality from MS / heat combination treatments. It is possible that the observed mortality is a combined consequence of contact and fumigant toxicity of the essential oil in combination with heat stress. High repellency of the MS also might have been effective in eliminating the refuge for termites during the heat treatment, causing the termites to be exposed to the lethal temperature. Further behavioral investigations would be necessary to elucidate this.

The approach investigated in this study, combining a volatile essential oil adjuvant with heat treatment, may be applicable in a range of scenarios where structural wood shielded by heat sinks is accessible for localized injection of an insecticidal essential oil. Although accessing such wood may not be feasible in some situations (e.g., wood located beneath a large bathroom fixture), other areas of structures might present straightforward opportunities for this combination approach. For instance, in structures with crawlspaces, sill plates and adjoining sections of wood may not heat easily during a heat treatment (Rust and Reierson 1998), while being relatively simple to access and treat with localized oil injections. The use of volatile essential oil adjuvants for heat treatment will be most effective in other, similar situations wherein wood is difficult to heat, but easy to access. By limiting essential oil application to these areas, the need to determine the precise locations of infestations can be avoided.

The overarching goal of the current studies was to improve heat treatment options for the western drywood termite by incorporating an insecticidal essential oil constituent. By overcoming the challenges associated with structural heat sinks, the proposed combination approach could provide an effective option for drywood termite control in situations where heat treatment by itself might prove inadequate.

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