*Journal of Economic Entomology*, XX(XX), 2024, 1–8
https://doi.org/10.1093/jee/toae101
Research

ENTOMOLOGICAL SOCIETY OF AMERICA
SHARING INSECT SCIENCE GLOBALLY
OXFORD

Household and Structural Insects

# Potential use of pinenes to improve localized insecticide injections targeting the western drywood termite (Blattodea: Kalotermitidae)

**Nicholas A. Poulos**[*], **Chow-Yang Lee**, **Michael K. Rust**, **Dong-Hwan Choe**

Department of Entomology, University of California, 900 University Avenue, Riverside, CA 92521, USA [*]Corresponding author, mail: nicholas.poulos@email.ucr.edu

Subject Editor: Arthur Appel

Received on 22 February 2024; revised on 23 April 2024; accepted on 3 May 2024

The western drywood termite, *Incisitermes minor* (Hagen), causes significant economic damage to wood structures in the United States of America, especially California. When infestation is not widespread, localized insecticide injections may be useful for remedial control. However, the extensive gallery structure of drywood termites and their tendency to aggregate at specific parts of the galleries can impact the efficacy of localized insecticide injection. Chemicals that attract termites from a distance may improve the localized insecticide injection by increasing the number of termites contacting the insecticide residues. Two volatile terpenes, α-pinene and β-pinene, commonly found in many coniferous timber trees, were applied to artificial termite galleries to determine if termites were attracted from their original aggregation site. Furthermore, we examined if adding these pinenes would improve the overall efficacy of some insecticide products for drywood termite control. Behavioral assay results showed that the treatment with pinenes increased the likelihood that drywood termites would leave their original aggregation site and contact the treated part of the gallery. When tested with the pesticide products applied in a small area away from the termite aggregation, β-pinene significantly accelerated the time course of mortality for the aqueous fipronil. The efficacy of disodium octaborate tetrahydrate dust was not influenced by the addition of pinenes. Implications for drywood termite management and future research directions are discussed.

*Key words: Incisitermes minor*, localized insecticide treatment, pinene, fipronil, disodium octaborate tetrahydrate

## Introduction

The western drywood termite, *Incisitermes minor* (Hagen) (Blattodea: Kalotermitidae), causes significant economic damage in its native range of the southwestern United States and northwestern Mexico (Harvey 1934, Cabrera and Scheffrahn 2001). Beyond its native range, *I. minor* has spread to other parts of the United States, for example, Hawaii, New York State, and Florida, and it is also reported in Canada, China, Japan, and Australia (Cabrera and Scheffrahn 2001, Evans et al. 2013, Scheffrahn 2019, Horwood and Lo 2022). Increased urbanization and globalization involving the movement of wood and wood-containing products worldwide, along with the cryptic lifestyle of *I. minor*, contribute to its status as an important structural pest in several parts of the world (Lewis and Forschler 2014). Western drywood termites typically nest and forage inside one piece of wood (or pieces that contact), with only winged reproductive (alates) leaving the nest for dispersal (Harvey 1934), making their detection and management challenging.

Remedial control options for drywood termite infestations can be categorized as whole-structure or localized treatment. Whole-structure treatment targets all infestations in a structure at once, while localized treatment targets infestations limited to single boards or a small group of boards. Structural fumigation with sulfuryl fluoride is the most common method for whole-structure treatment (Lewis and Forschler 2014, Zilberman and Lewis 2024). However, structural fumigation for drywood termite control has several drawbacks, including its high cost, disruptiveness (in California, structural fumigators must implement the California Aeration Plan), and the lack of residual protection (Cabrera and Scheffrahn 2001). In addition, sulfuryl fluoride is classified as a significant greenhouse gas (Papadimitriou et al. 2008, Mühle et al. 2009), and its use for structural fumigation might undergo more regulatory scrutiny in the future (Barreau et al. 2019, Gaeta et al. 2021).

When infestations are relatively small and readily accessible, localized insecticide injection, also known as 'drill-and-treat' or 'spot' treatment, is a useful control option with residual protection (Cabrera and Scheffrahn 2001, Lewis and Forschler 2014). It involves injecting insecticides into infested wood through evenly-spaced drill holes (often in a diamond pattern), some of which might intersect existing termite galleries (Scheffrahn et al. 1997). When accessible, kick-out holes (for fecal pellets) provide direct access to active drywood termite galleries (Cabrera and Scheffrahn 2001). Since the termites must contact or ingest the injected insecticide to be killed, the success of localized insecticide injection largely depends upon the ability to locate active infestations within the structure (Cabrera and Scheffrahn 2001). However, the extensive gallery systems in mature colonies of the drywood termite and its aggregation behavior in certain parts of the galleries (Minnick et al. 1973, Cabrera and Rust 1996, 2000) might limit the injected insecticide from reaching all the termites at lethal doses, substantially impacting control efficacy (Scheffrahn et al. 1997, Woodrow et al. 2006). The possibility of leaving pockets of survivors and subsequent reestablishment of an infestation is one of the most common concerns for the localized insecticide injection method.

The concept of attract-and-kill may have the potential to provide a solution for this challenge. The attract-and-kill method involves applying a pheromone or other chemical attractant and an insecticide (killing agent) (Gregg et al. 2018). The attractant lures the target insect pest to the insecticide deposit, and the insect picks up a lethal dose upon contact (Charmillot et al. 2000, Gregg et al. 2018). If the locally injected insecticide deposits can attract drywood termites from a distance (within their gallery), control efficacy might be maximized even when the injection does not provide complete coverage of the target termite gallery system. To test this idea, it is important to find effective attractants that would lure termites away from their aggregations to treated areas, increasing the overall exposure level of the termite colony to the insecticide residue.

Galleries of mature *I. minor* colonies are typically found in the sapwood of dry, sound wood (without decay) (Harvey 1934). Differences in physical and chemical characteristics between sapwood and heartwood might be responsible for this apparent preference (Rust et al. 1979, Sajap and Sahri 1983, Kasseney et al. 2011). Based on a series of choice tests involving 10 different kinds of timbers, Indrayani et al. (2007) concluded that sapwood and heartwood of Norway spruce (*Picea abies* Karsten) were most preferred by *I. minor* workers (pseudergates). Their choice feeding data also indicated a slight trend of preference for the sapwood of Norway spruce over its heartwood. Himmi et al. (2014) reported that founding *I. minor* reproductive preferred the sapwood over the heartwood of Sitka spruce (*Picea sitchensis* Bong. Carriere) for their initial colonization. Since Sitka spruce heartwood does not show any deterrence effect on *I. minor* (Himmi et al. 2016), these observations suggest that *I. minor's* preference for sapwood over heartwood of spruce timber (*Picea* spp.) is possibly related to the presence of attractant(s) in the sapwood rather than the presence of deterrent(s) or repellent(s) in the heartwood.

Based on an aeration study investigating the total volatile organic compound (TVOC) concentration of sapwood and heartwood, Czajka et al. (2020) reported that sapwood of Norway spruce (*P. abies*) releases 2–3 times more TVOC than its heartwood. Two volatile monoterpenes, α-pinene, and β-pinene, are the most abundant organic compounds found in the headspace of Norway spruce. The volatiles of Norway spruce sapwood contained more α-pinene than those of heartwood while β-pinene was present only in the sapwood volatiles (Czajka et al. 2020). Wajs et al. (2007) reported a similar finding with Norway spruce using solid-phase microextraction combined with gas chromatography-mass spectrometry.

In this study, we tested if treatment with α- or β-pinene could affect the behavior of *I. minor* workers within simulated wooden gallery systems. By developing channel arena and T-maze arena assays, we explored if α- or β-pinene added to specific parts of the wooden gallery could lure *I. minor* workers away from their existing aggregation, increasing the chance of contacting the treated area. Based on the initial behavioral investigation, we also tested the incorporation of α- or β-pinene with selected insecticide residues, which would affect mortality trends in *I. minor* colonies and overall control efficacy compared to the insecticide-only standard treatments.

## Materials and Methods

### Termites

Western drywood termites, *I. minor*, were extracted from several pieces of infested wood collected in various locations in Riverside, California. Species identification was based on the morphology of alates (when present), soldiers, and fecal pellets. Each colony was stored in a plastic container (30.5 × 21.6 × 6.4 cm) with small pieces of basswood (150 × 100 × 3.2 mm; Midwest Products Co., Inc., Darien, Illinois) as a food source. A couple of small openings were made on the sides of the containers for ventilation, and the containers were placed within a larger plastic box (40.6 × 22.9 × 27.9 cm) containing a saturated NaCl solution to maintain 75% RH within the larger box (Winston and Bates 1960). The stock colony boxes were kept in a growth chamber (Thermo Fisher Scientific, Waltham, Massachusetts) at 26°C without light. To minimize the chance of using termites that were damaged during the collection process, the collected termites were left undisturbed for at least 7 days and up to 4 wk, and the surviving termites at the end of this period were used in the experiments. One or 2 different populations were randomly chosen to set up each experimental trial. A random mixture of pseudergates and nymphs, ≈ 5 mm in length, was used for the experiments.

### Chemicals

Two monoterpenes, α-pinene (98%, Sigma-Aldrich, St. Louis, Missouri) and (-)-β-pinene (≥ 99%, Sigma-Aldrich), were individually tested for their effects on drywood termite behavior. Methanol was used to dissolve the pinenes at 0.1 mg/ml concentration. The concentration and application rate of the methanol preparation of pinenes in the bioassays were based on a series of preliminary observations with individual and groups of termites using a range of concentrations, 0.01–1.0 mg/ml (unpublished data). Neat methanol served as a solvent-only control.

### Channel Arena Bioassay

A channel arena was developed to examine the behavioral effect of α-pinene and β-pinene on a group of *I. minor* (20 termites) within a simplified termite gallery system. It was made from 2/4 Douglas fir boards (25 × 8.9 × 3.81 cm). Each board was cut in half horizontally, producing 2 flat pieces (top and bottom) with identical dimensions (25 × 8.9 × 1.9 cm). A channel (19.8 × 1.3 cm, 0.6 cm in depth) was routed in the bottom piece along the centerline, simulating a termite gallery (Fig. 1). A strip of clear acrylic sheet (2.5 × 22 × 0.2 cm) was held in place over the channel with masking tape, permitting observation of the termites in the channel. The top piece of the arena was placed on top of the acrylic panel to keep the channel dark during the experiment.

To establish the "initial-aggregation zone," 20 termites were confined to a 5-cm zone at 1 end of the channel for 24 h using a

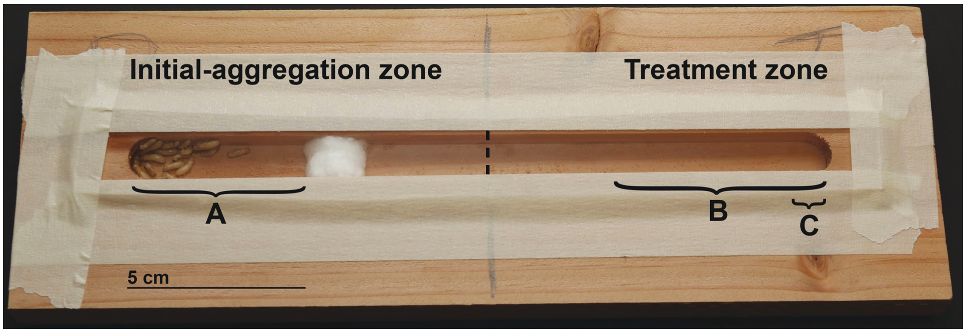

**Fig. 1.** A channel arena. A piece of cotton ball was used to confine the termites to a 5-cm section of the channel for 24 h establishing the initial-aggregation zone (A). A ≈ 6-cm section of the treatment zone (B) was used to apply a pinene dissolved in methanol (or methanol only for control). A ≈ 1-cm section at the end of the treatment zone (C) shows the area where insecticide treatments were made for the insecticide bioassays.

cotton plug (Fig. 1). After establishing the initial-aggregation zone, 100 µl of the methanol preparation containing a pinene (either α- or β-pinene) or clean methanol was applied to the opposite end of the channel (average spread of 6 cm). This resulted in an application rate of 1.3 µg/cm$^2$ for α- or β-pinene. The half of the channel containing this treated area served as the 'treatment zone' (Fig. 1). After the solvent evaporated for 3 min, the cotton plug was removed and the arenas were assembled. The arenas were maintained at 21–24°C and 31%–36% RH in complete darkness. After 24 h, the number of termites in the treatment zone was counted under red light (110 lux, Tacklife; Levittown, New York) to minimize any disruption to the termites during observation (Cabrera and Rust 1996). Each of the treatments and control were replicated 10 times. Orientation of the treatment and control zones was randomized to prevent any directional bias.

## T-maze Bioassay

A T-maze bioassay was developed to study the effects of α- or β-pinene on the choice behavior of *I. minor* within a simplified termite gallery system. The T-maze consisted of 3 basswood blocks (2.54 × 2.54 × 10.16 cm). One block served as a central acclimation block, while the other 2 served as lateral choice blocks (Fig. 2). All blocks were drilled along the centerline to form longitudinal tunnels (8.9 cm in depth, 0.95 cm in diameter), which ended near the opposite end of the block (about 1.2 cm away from the opposite end of the block). For the acclimation block, a through hole (0.95 cm in diameter) was drilled to add a cross tunnel, perpendicularly intersecting the longitudinal tunnel at its closed end. This cross tunnel provided connection points between the acclimation block and 2 choice blocks.

Twenty termites from the stock colony were placed in the tunnel of the acclimation block and confined there for 24 h by sealing the holes with rubber stoppers. Two choice blocks were randomly assigned for treatment (treated block) or no treatment (untreated block). The furthest end of the gallery in the treated block was treated with 100 µl of methanol preparations (0.1 mg/ml α- or β-pinene) or clean methanol (solvent-only control) using a glass microcapillary tube (Drummond Scientific Company, Broomall, Pennsylvania) while positioning the block vertically during the treatment. The treated block was left to dry for 5 min. After removing the 2 rubber stoppers from the cross tunnel of the acclimation block, the treated and untreated choice blocks were connected to the acclimation block using a double-sided working tape (Hippie Crafter, Miami Beach, Florida). The T-mazes were stored in a growth chamber at 26°C and 20%–26% RH in complete darkness. After 24 h, T-mazes were disassembled, and numbers of termites in each of the 3 blocks (2 choice blocks and 1 acclimation block) were recorded. Treatments and solvent-only control were replicated 10 times. The positions of the treated and untreated blocks alternated for each replication to prevent any directional bias.

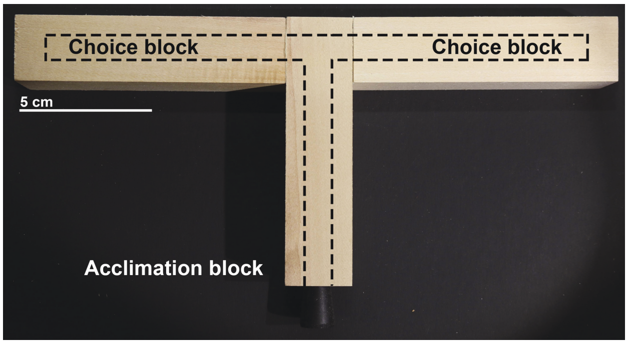

**Fig. 2.** An assembled T-maze showing approximate arrangement of internal tunnels.

## Insecticide Bioassay

The effect of pinenes on the efficacy of insecticide residue was evaluated using the channel arena bioassay design (Fig. 1). Even though the T-maze would simulate slightly more complex gallery systems than the channel arena, the latter was selected for the insecticide bioassay due to its advantage of permitting repeated observations of termites over time without the need of disassembling the arena. The experimental protocol was identical to the method previously described for the channel arena bioassay except for applying an insecticide product at the end of the treatment zone (Fig. 1).

The following insecticide products were used in the experiment: Termidor SC (9.1% fipronil; BASF, Research Triangle, North Carolina) and Tim-Bor (98% disodium octaborate tetrahydrate [DOT]; Nisus Corporation, Rockford, Tennessee). They were selected based on a laboratory study showing relatively high efficacy for drywood termite control (Rust and Venturina 2009). Both products are currently registered for drywood termite control.

The termites were first confined in the initial-aggregation zone. For this process, the acrylic sheet covered only ≈ 2/3 of the channel containing the initial-aggregation zone, leaving the ≈ 1/3 of the channel (with treatment zone) uncovered. For fipronil, 10 µl of 0.06% aqueous suspension was applied in a small area (1.43 cm$^2$) at the end of the treatment zone using a glass microcapillary tube and allowed to dry for 24 h. After 24 h, the methanol preparation of α- or β-pinene was applied at the end of the treatment zone and allowed to dry for 3 min. Subsequently, the cotton plug in the channel was removed and the acrylic sheet was slid to cover the entire arena and taped in place. The 24-h drying period for the treatment zone was necessary to avoid uncontrolled effects that water (from aqueous fipronil preparation) might have on termite behavior.

DOT was applied as dust. Based on the minimum label rate recommended for drill-and-injection treatment (130 g/m$^2$), 13 mg/cm$^2$ was used as the application rate. To test DOT dust with the volatile attractant, the methanol preparation of α- or β-pinene was first applied at the end of the treatment zone, and the solvent was allowed to evaporate for 3 min. Subsequently, DOT dust was applied at the end of the treatment zone (1.43 cm$^2$) and spread evenly with the tip of a clean glass microcapillary tube. This specific treatment order was necessary to prevent methanol from impacting the physical properties of the DOT dust.

Two different control groups were included in the experimental design: 1 without insecticide, and the other with insecticide. Both control groups received clean methanol only (no pinene). The arenas were maintained at 21–24°C and 31%–36% RH in complete darkness. Observations were made under red light every 24 h for 14 days. Termites were recorded as dead or alive. Due to the acrylic panel seal, touching the termites to assess mortality was not possible during the bioassay. Instead, the acrylic panel above the termites was gently tapped, and the termites without any movement for ≈ 5 s were considered dead. Each treatment and control were replicated 9–14 times. Orientation of the treatment and control zones was randomized to prevent any directional bias.

### Statistical Analyses

All count data were converted to percentage values for analyses except for the Kaplan–Meier survival analysis. Due to the violation of normality (T-maze assay) and/or homogeneity of variance assumptions (channel arena and T-maze assays), the data were analyzed using nonparametric methods. For the channel arena assay, data from the solvent only and pinene treatments were compared with a Kruskal–Wallis test followed by Dunn posthoc all-pairwise comparisons. For the T-maze assay, a Wilcoxon signed-rank test was used to compare the percentage of termites found in the untreated and treated blocks. For the insecticide bioassays, data were first analyzed with a Kaplan–Meier survival analysis (Kaplan and Meier 1958). The distribution of survival times of termites was described using the survivorship function $S(t)$, the probability of an individual termite surviving past a given time point, $t$ (in days). Log-rank tests (Peto and Peto 1972) were used for overall comparisons among survival curves and subsequent multiple comparisons using the Holm–Sidak method (Šidák 1967). The final cumulative mortality data (day 14) were normalized using an arcsine transformation and subsequently analyzed with a 1-way analysis of variance (ANOVA) followed by Tukey's test. Additionally, to understand the mortality caused by insecticides, the average final cumulative mortality data were corrected using Abbott's formula (Abbott 1925). All statistical analyses were performed in Sigma Plot ver.14.5 (Systat Software, San Jose, California).

## Results

### Channel Arena Bioassay

When methanol was used for treatment, 14.5% ± 5.1% (mean ± standard error of the mean [SEM]) of termites were found in the treatment zone after 24 h. In contrast, 55% ± 9.3% or 58% ± 6.3% of termites were found in the treatment zone when it was treated with α- or β-pinene, respectively (Fig. 3) (Kruskal–Wallis test: $H = 14.40$; df = 2; $P < 0.0001$). Dunn's pairwise comparisons showed that the percentage of termites in the treatment zone from α-pinene and β-pinene treatments were significantly different from that of solvent only ($Z = 3.07$; $P = 0.002$ for α-pinene and $Z = 3.44$; $P < 0.001$ for β-pinene). However, data from α-pinene and β-pinene treatments were not significantly different from each other ($Z = 0.37$; $P = 0.712$).

### T-maze Bioassay

When methanol was used to treat 1 of the choice blocks, similar numbers of termites were found in the untreated and treated blocks of the maze at 24 h (Wilcoxon signed-rank test: $W = 3$; $P = 0.844$). On average, 15.5% ± 10.2% and 20% ± 11.8% of the termites were found in the untreated and treated blocks, respectively (Fig. 4A). When α-pinene was used to treat 1 of the choice blocks, 7.0% ± 7.0% and 45.5% ± 14.1% of the termites were found in the untreated and treated blocks, respectively (Fig. 4B). However, the difference was not statistically significant (Wilcoxon signed-rank test: $W = 19$; $P = 0.063$). When β-pinene was used for treatment, 4.5% ± 3.5% of the termites were found in the untreated block, while 54.5% ± 14.8% were found in the treated block. This difference was statistically significant (Wilcoxon signed-rank test: $W = 28$; $P = 0.016$) (Fig. 4C).

### Insecticide Bioassay for Fipronil

Overall, a significant difference among the survival curves was found (log-rank test: $\chi^2 = 249.39$; df = 3; $P < 0.001$) (Fig. 5A). Pairwise comparisons indicated all survival curves were significantly different from each other.

The final cumulative mortality levels differed among groups (ANOVA: $F = 37.91$; df = 3, 30; $P < 0.001$) (Fig. 6A). Between the treatments with pinene, only fipronil + β-pinene (96.5% ± 2.1%) was significantly different from the fipronil only control (76.5% ± 5.7%) ($P = 0.006$). The difference between fipronil only and fipronil + α-pinene (85% ± 5.7%) was not significant ($P = 0.593$). The α- and β-pinene fipronil treatments had similar mortality levels ($P = 0.129$). The solvent only control had 5.0% ± 1.6% mortality, which was significantly different from all other treatments (Fig. 6A). When corrected by Abbott's formula, the final cumulative mortalities were

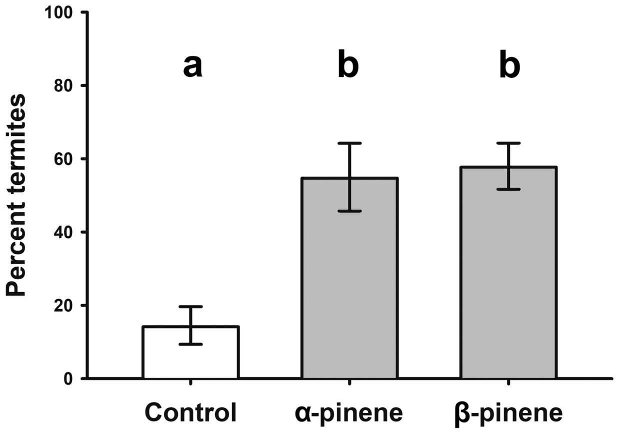

**Fig. 3.** Percent (mean ± SEM) *I. minor* found in the treatment zone of the channel following treatment with solvent only (control), α-pinene or β-pinene. Bars with different letters are statistically different from each other (α = 0.05; Dunn all-pairwise comparison).

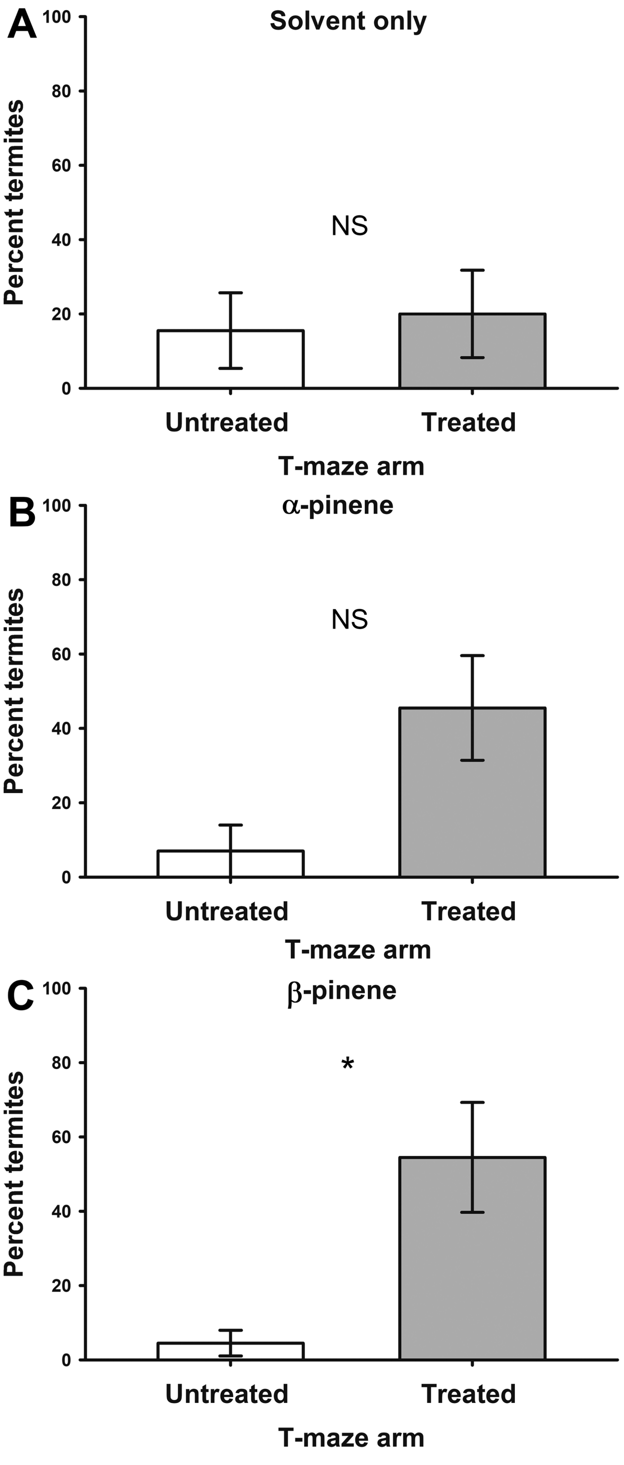

**Fig. 4.** Percent (mean ± SEM) *I. minor* found in choice blocks of the T-maze. The treated block received a treatment with solvent only (A), α-pinene (B), or β-pinene (C). The asterisk indicates a statistically significant difference (α = 0.05; Wilcoxon signed-rank test).

75.26%, 84.21%, and 96.32% for fipronil only, fipronil + α-pinene, and fipronil + β-pinene, respectively.

### Insecticide Bioassay for DOT

Overall, a significant difference among the survival curves was found (log-rank test: $\chi^2 = 104.47$; df = 3; $P < 0.001$) (Fig. 5B). All pairwise comparisons indicated all survival curves were significantly different from each other, except DOT only and DOT + β-pinene ($P = 0.733$).

The final cumulative mortality differed among groups (ANOVA: $F = 9.394$; df = 3,35; $P < 0.001$) (Fig. 6B). However, day 14 mortality levels were statistically similar between DOT only (70% ± 9.6%), DOT + α-pinene (55% ± 6.4%), and DOT + β-pinene (68.93% ± 7.6%). The solvent only control mortality (5% ± 1.6%), was significantly different from all other treatments (Fig. 6B). When corrected by Abbott's formula, the final cumulative mortalities were 68.42%, 52.63%, and 67.29% for DOT only, DOT + α-pinene, and DOT + β-pinene, respectively.

## Discussion

Pinenes provide a variety of functions across insect systems. Repellent properties of pinenes are known for several insect species. For example, β-pinene has a repellent activity against the red flour beetle, *Tribolium castaneum* (Herbst) (Pajaro-Castro et al. 2017) and the German cockroach, *Blatella germanica* (L.) (Liao et al. 2017). Also, α- and β-pinene have been identified as the major active components in the alarm pheromone of the vetch aphid, *Megoura viciae* (Buckton) (Song et al. 2021). Additionally, pinenes can function as attractants in some insect species. Both pinenes have shown properties as an attractant for various coleopterans (Metcalf and Kogan 1987, Zhao et al. 2020, Kelsey et al. 2023) and dipterans (Stappen et al. 2021). Furthermore, both α- and β-pinene show attractive and alarming effects on subterranean termites, *Reticulitermes* spp. (Reinhard et al. 2003).

The channel arena and T-maze bioassays (no insecticide) demonstrated that small amounts of pinenes applied within the arenas caused *I. minor* to move away from their initial aggregations and contact the treated areas. While the result of the channel arena bioassay may simply suggest that the pinene treatments made the termites leave their initial aggregation, the result from the T-maze bioassay clearly indicate that the termites preferred the area treated with pinenes. However, since the current study employed 1-time observation after 24 h (i.e., no continuous observation of insect behavior), it remains unclear how the treatment influenced the termites' behavior within the wooden arena over the 24-h period. α- and β-Pinene have a vapor pressure of 4.75 mm Hg and 2.93 mm Hg at 25°C, respectively (Daubert 1989). Given the volatility of pinenes (Silva et al. 2004), it is possible that some amount of pinenes volatilized and diffused within the galleries, reaching the termites in the initial aggregation and subsequently impacting their behaviors. It is also possible that arrestant response might be, at least in part, responsible for the final location of termites in the pinene treatments (i.e., more termites were found in the treated zone because they slowed down or stopped their locomotion when they got close to the treated zone or contacted the treated surfaces).

α- and β-Pinene are 2 isomers of pinene, a group of double-bond containing monoterpenes (Silva Rivas et al. 2012). Although α- and β-pinene performed similarly in the channel arena bioassay (no insecticide), it is important to note that there was an apparent (and statistically significant) difference between the 2 based on the T-maze results. Unlike the β-pinene treatment, the number of termites found in untreated and treated blocks was not significantly different when α-pinene was used for treatment. This result may indicate the inherent difference between these 2 compounds regarding their impact on drywood termite behavior. The nonsignificant difference between fipronil only and fipronil + α-pinene in the insecticide bioassay (fipronil, see below) also supports this possibility. Slight differences between isomers, and even enantiomeric differences within an isomer,

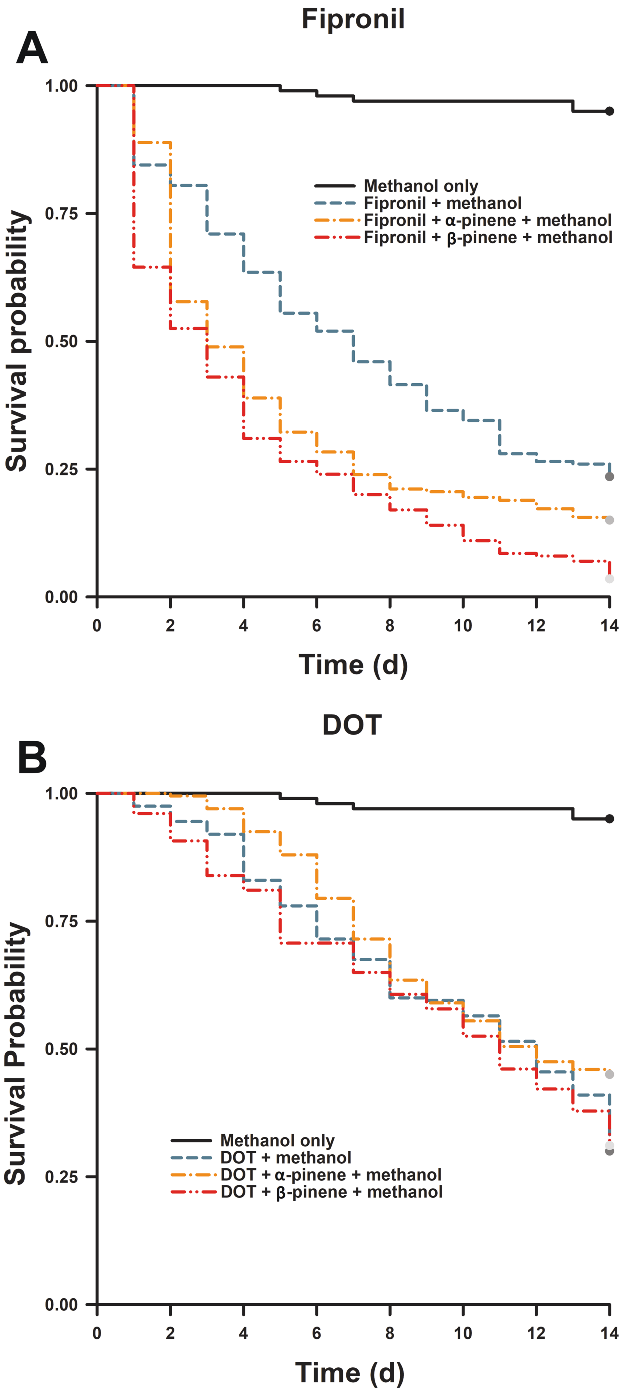

**Fig. 5.** Kaplan—Meier survival curves of *I. minor* from fipronil (A) and DOT (B) insecticide bioassays. Data from the methanol-only control (without insecticide) were used for both groups.

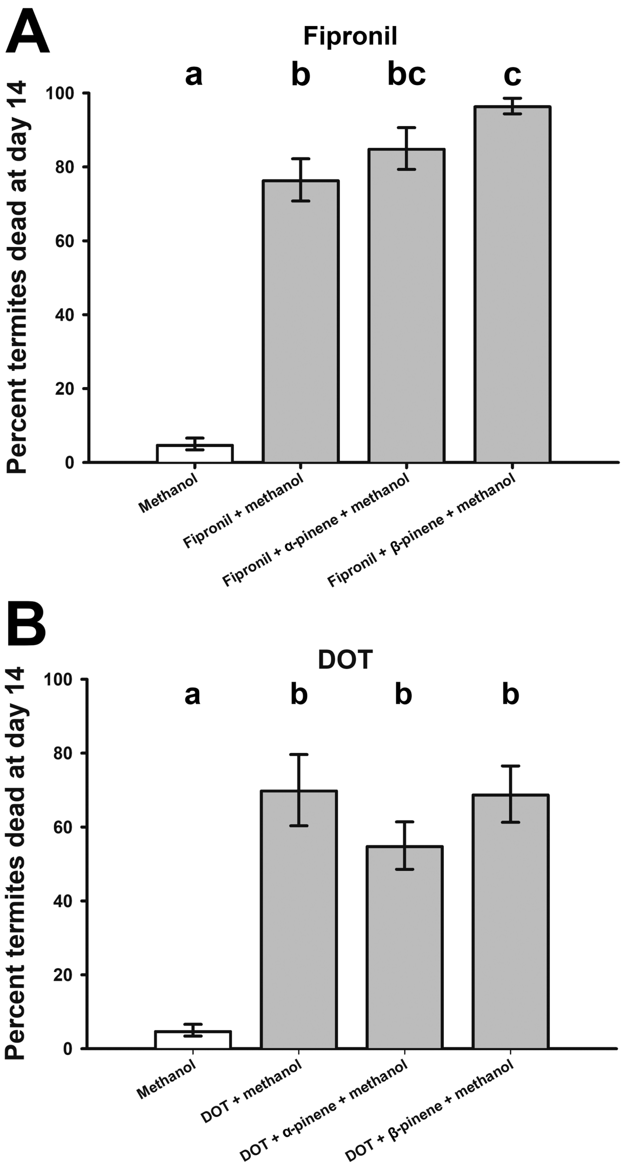

**Fig. 6.** Percent (± SE) mortality of *I. minor* from fipronil (A) and DOT (B) insecticide bioassays at day 14. Data from the methanol-only control (without insecticide) were used for both groups. Bars with different letters are statistically different from each other ($\alpha = 0.05$; Tukey's test).

can result in drastically different effects on organisms (Reinhard et al. 2003, Silva Rivas et al. 2012).

Pinenes are a terpene commonly found in nature and represent 75%–90% of the essential oils found in conifers (Loza-Tavera 1999, Vespermann et al. 2017). Since the wooden arenas made from Douglas fir and basswood may already contain 1 or both pinenes, finding the response of termites to the pinene treatment was particularly intriguing. It is possible that the termites responded to the presence of higher concentrations of the pinenes caused by the treatment. Eberhardt et al. (2009) showed a significant decline of β-pinene levels over time in pine stumps, likely due to volatilization, oxidation and isomerization. Given that both pinenes are volatile, it is likely that the structural wood (including the Douglas fir boards and basswood blocks used to construct the wooden arenas) would lose the pinenes below a certain level over time. The treatment may have essentially 'replenished' the pinene levels in the treated area, resulting in behavioral changes of *I. minor* within the gallery.

The efficacy of aqueous fipronil treatment was significantly improved by adding β-pinene. The termites were killed more quickly, and the final mortality was higher than the fipronil-only treatment. This shows the potential utility of β-pinene as an adjuvant to improve the efficacy of localized insecticide injections for drywood termite control. α-Pinene may have a similar effect on aqueous fipronil treatment, but we did not detect any statistical significance. In contrast, the pinene treatment did not influence the efficacy of DOT dust treatment. Fipronil and DOT differ in their mode of action. For example, DOT acts as a stomach poison after being ingested while grooming (Rust and Reierson 1981), while fipronil blocks the γ-aminobutyric acid-gated chloride channel, primarily acting as a contact poison (Hainzl and Casida 1996). To find optimal combinations for localized insecticide injection, more work must be done to tease apart the relationships that different active ingredients and formulations have with β-pinene.

Ebeling (1975) recommended drilling holes spaced by 30 cm (≈ 12 inches) to provide access to the galleries within the infested wood for localized insecticide injection to control drywood termites. More recent studies employed 7.6–12.7 cm (3–5 inches) or 45.7 cm (18 inches) intervals between drill holes (arranged in a diamond pattern) for field and laboratory experiments, respectively (Lewis et al. 2009, Rust and Venturina 2009). However, there is no industry standard regarding the arrangement of these drill holes. Various factors such as the active ingredient or formulation used, product label instructions, extent of infestation, and location of the infested wood can also influence the optimal distance between drill holes (Ebeling 1975, Scheffrahn et al. 1997). Therefore, the distance between drill holes is at the discretion of the technician who performs the treatment, and it may vary from case to case. Incorporating an attractant, such as β-pinene, in localized insecticide injection could allow for wider spacing between drill holes without negatively impacting the efficacy of the treatment. This may help improve the cost-effectiveness of localized insecticide injections by reducing the time and labor needed for the treatment. Additionally, although not explicitly tested in the present study, it is possible that the incorporation of an attractant such as β-pinene for localized insecticide injection could also help reduce the amount of insecticide product needed to achieve a desirable level of control. Further study is warranted to explore these aspects.

## Acknowledgments

We thank Kathleen Campbell, Ho Eun Park, and Kevin Giloni (University of California, Riverside) for their assistance in this study. Special thanks to Dr. Thomas Perring (University of California, Riverside) for providing many of the termites used in this study.

## Funding

The project was funded in part by the intramural research program of the U.S. Department of Agriculture, National Institute of Food and Agriculture (Hatch project accession number: 7003981). The funding agency does not necessarily recommend or endorse any opinion, commercial product, or trade name used in the article. We also thank the Carl Strom/Western Exterminator Scholarship program for partial financial support.

## Author Contributions

Nicholas Poulos (Conceptualization [Equal], Data curation [Lead], Formal analysis [Lead], Investigation [Lead], Methodology [Equal], Project administration [Lead], Visualization [Lead], Writing—original draft [Lead]), Chow-Yang Lee (Conceptualization [Supporting], Formal analysis [Supporting], Methodology [Supporting], Writing—review & editing [Supporting]), Michael Rust (Conceptualization [Supporting], Formal analysis [Supporting], Methodology [Supporting], Writing—review & editing [Supporting]), and Dong-Hwan Choe (Conceptualization [Equal], Formal analysis [Supporting], Funding acquisition [Lead], Methodology [Equal], Resources [Lead], Supervision [Lead], Validation [Lead], Writing—review & editing [Lead])

## References

Abbott WS. A method of computing the effectiveness of an insecticide. J Econ Entomol. 1925:18(2):265–267. https://doi.org/10.1093/jee/18.2.265a

Barreau T, Hoshiko S, Kreutzer R, Smorodinsky S, Talarico J. Sulfuryl fluoride poisonings in structural fumigation, a highly regulated industry-potential causes and solutions. Intern J Environ Res Public Health. 2019:16(11):2026. https://doi.org/10.3390/ijerph16112026

Cabrera BJ, Rust MK. Behavioral responses to light and thermal gradients by the western drywood termite (Isoptera: Kalotermitidae). Environ Entomol. 1996:25(2):436–445. https://doi.org/10.1093/ee/25.2.436

Cabrera BJ, Rust MK. Behavioral responses to heat in artificial galleries by the western drywood termite (Isoptera: Kalotermitidae). J Agric Urban Entomol. 2000:17(3):157–171.

Cabrera BJ, Scheffrahn RH. 2001. Western drywood termite, *Incisitermes minor* (Hagen) (Insecta: Isoptera: Kalotermitidae). UF/IFAS Extension EENY. 248:1–7.

Charmillot PJ, Hofer D, Pasquier D. Attract and kill: a new method for control of the codling moth *Cydia pomonella*. Entomol Exp Appl. 2000:94(2):211–216. https://doi.org/10.1046/j.1570-7458.2000.00621.x

Czajka M, Fabisiak B, Fabisiak E. Emission of volatile organic compounds from heartwood and sapwood of selected coniferous species. Forests. 2020:11(1):92. https://doi.org/10.3390/f11010092

Daubert TE. Physical and thermodynamic properties of pure chemicals: data compilation. New York (US): Design Institute for Physical Property Data; 1989.

Ebeling W. Urban Entomology. Los Angeles (CA): University of California Press; 1975. p. 161–162.

Eberhardt TL, Sheridan PM, Mahfouz JM. Monoterpene persistence in the sapwood and heartwood of longleaf pine stumps: assessment of differences in composition and stability under field conditions. Can J For Res. 2009:39(7):1357–1365. https://doi.org/10.1139/x09-063

Evans TA, Forschler BT, Grace JK. Biology of invasive termites: a worldwide review. Annu Rev Entomol. 2013:58:455–474. https://doi.org/10.1146/annurev-ento-120811-153554

Gaeta D, Vimont I, McKain K, Crotwell M, Zhang M, Miller B, Muhle J, Miller S. Greater Los Angeles Area dominates US emissions of sulfuryl fluoride, a potent greenhouse gas. Paper Presented at AGU Fall Meeting. New Orleans (LA); 2021.

Gregg PC, Del Socorro AP, Landolt PJ. Advances in attract-and-kill for agricultural pests: beyond pheromones. Annu Rev Entomol. 2018:63(1):453–470. https://doi.org/10.1146/annurev-ento-031616-035040

Hainzl D, Casida JE. Fipronil insecticide: novel photochemical desulfinylation with retention of neurotoxicity. Proc Natl Acad Sci U S A. 1996:93(23):12764–12767. https://doi.org/10.1073/pnas.93.23.12764

Harvey PA. Life history of *Kalotermes minor*. In: Kofoid CA, editor. Termites and Termite Control. 2nd ed. Berkeley (CA): University of California Press; 1934. p. 217–233.

Himmi SK, Yoshimura T, Yanase Y, Oya M, Torigoe T, Imazu S. X-ray tomographic analysis of the initial structure of the royal chamber and the nest-founding behavior of the drywood termite *Incisitermes minor*. J Wood Sci. 2014:60(6):453–460. https://doi.org/10.1007/s10086-014-1427-x

Himmi S, Yoshimura T, Yanase Y, Oya M, Torigoe T, Akada M, Imadzu S. Nest-Gallery Development and Caste Composition of Isolated Foraging Groups of the Drywood Termite, Incisitermes minor (Isoptera: Kalotermitidae). *Insects*. 2016:7(3):38–38. https://doi.org/10.3390/insects7030038

Horwood M, Lo N. First detection and eradication of a structural infestation by western drywood termite, *Incisitermes minor* (Hagen)(Isoptera: Kalotermitidae). Austral Entomol. 2022:61(3):378–383. https://doi.org/10.1111/aen.12615

Indrayani Y, Yoshimura T, Yanase Y, Imamura Y. Feeding responses of the western dry-wood termite *Incisitermes minor* (Hagen) (Isoptera: Kalotermitidae) against ten commercial timbers. J Wood Sci. 2007:53(3):239–248. https://doi.org/10.1007/s10086-006-0840-1

Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc. 1958:53(282):457–481. https://doi.org/10.1080/01621459.1958.10501452

Kasseney BD, Deng T, Mo J. Effect of wood hardness and secondary compounds on feeding preference of *Odontotermes formosanus* (Isoptera: Termitidae). J Econ Entomol. 2011:104(3):862–867. https://doi.org/10.1603/ec10216

Kelsey RG, Westlind DJ, Gaylord ML. Red turpentine beetle primary attraction to β-pinene or 3-carene (with and without ethanol) varies in western US pine forests. Agric For Entomol. 2023:25(1):111–118. https://doi.org/10.1111/afe.12535

Lewis VR, Forschler B. Management of drywood termites: past practices, present situation and future prospects. In: Dhang P, editor. Urban Insect Pests: Sustainable Management Strategies. Boston (MA): CABI; 2014. p. 130–153. https://doi.org/10.1079/9781780642758.0130

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

Liao S, Liu Y, Si H, Xiao Z, Fan G, Chen S, Wang P, Wang Z. Hydronopylformamides: Modification of the naturally occurring compound (-)-β-pinene to produce insect repellent candidates against *Blattella germanica*. Molecules. 2017:22(6):1004. https://doi.org/10.3390/molecules22061004

Loza-Tavera H. Monoterpenes in essential oils: biosynthesis and properties. In: Shahidi F, Kolodziejczyk P, Whitaker JR, et al., editors. Chemicals via higher plant bioengineering. Boston, MA: Springer; 1999. p. 49–62. https://doi.org/10.1007/978-1-4615-4729-7_5

Metcalf RL, Kogan M. Plant volatiles as insect attractants. Crit Rev Plant Sci. 1987:5(3):251–301. https://doi.org/10.1080/07352688709382242

Minnick DR, Kerr SH, Wilkinson RC. Humidity behavior of the drywood termite *Cryptotermes brevis*. Environ Entomol. 1973:2(4):597–602. https://doi.org/10.1093/ee/2.4.597

Mühle J, Huang J, Weiss RF, Prinn RG, Miller BR, Salameh PK, Harth CM, Fraser PJ, Porter LW, Greally BR, et al. Sulfuryl fluoride in the global atmosphere. J Geophys Res Atmos. 2009:114(D5):D05306. https://doi.org/10.1029/2008JD011162

Pajaro-Castro N, Caballero-Gallardo K, Olivero-Verbel J. Neurotoxic effects of linalool and β-pinene on *Tribolium castaneum*. Molecules. 2017:22(12):2052. https://doi.org/10.3390/molecules22122052

Papadimitriou VC, Portmann RW, Fahey DW, Mühle J, Weiss RF, Burkholder JB. Experimental and theoretical study of the atmospheric chemistry and global warming potential of $SO_2F_2$. J Phys Chem A. 2008:112(49):12657–12666. https://doi.org/10.1021/jp806368u

Peto R, Peto J. Asymptotically efficient rank invariant test procedures. J Royal Stat Soc Ser A. 1972:135(2):185–198. https://doi.org/10.2307/2344317

Reinhard J, Quintana A, Sreng L, Clément JL. Chemical signals inducing attraction and alarm in European *Reticulitermes* termites (Isoptera, Rhinotermitidae). Sociobiol. 2003:42(3):675–692.

Rust ME, Reierson DA, Scheffrahn RH. Comparative habits, host utilization and xeric adaptations of the Southwestern drywood termites, *Incisitermes fruticavus* Rust and *Incisitermes minor* (Hagen)(Isoptera: Kalotermitidae). Sociobiol. 1979:4(2):239–255.

Rust MK, Reierson DA. Attraction and performance of insecticidal baits for German cockroach control. Int Pest Control. 1981:23(4):106–109.

Rust MK, Venturina J. 2009. Evaluation of chemical localized treatment for drywood termite control. Progress report prepared for the California Structural Pest Control Board, Sacramento, CA. https://pestboard.ca.gov/howdoi/research/2009_drywood_rpt.pdf.

Sajap AS, Sahri MH. Responses to wood and wood extractives of *Neobalanocarpus heimii* and *Shorea ovalis* by the drywood termite, *Cryptotermes cynocephalus* (Isoptera: Kalotermitidae). Pertanika. 1983:6(3):28–31.

Scheffrahn RH. 2019. UF termite database. University of Florida termite collection. [accessed 2024 Feb 4]. https://termitediversity.org.

Scheffrahn RH, Su NY, Busey P. Laboratory and field evaluations of selected chemical treatments for control of drywood termites (Isoptera: Kalotermitidae). J Econ Entomol. 1997:90(2):492–502. https://doi.org/10.1093/jee/90.2.492

Šidák Z. Rectangular confidence regions for the means of multivariate normal distributions. J Am Stat Assoc. 1967:62(318):626–633. https://doi.org/10.1080/01621459.1967.10482935

Silva CM, Cláudio Filho A, Quadri MB, Macedo EA. Binary diffusion coefficients of α-pinene and β-pinene in supercritical carbon dioxide. J Supercrit Fluids. 2004:32((1-3):167–175. https://doi.org/10.1016/j.supflu.2004.01.003

Silva Rivas AC, Lopes PM, de Azevedo Barros MM, Costa Machado DC, Alviano CS, Alviano DS. Biological activities of α-pinene and β-pinene enantiomers. Mol. 2012:17((6):6305–6316. https://doi.org/10.3390/molecules17066305

Song X, Qin YG, Yin Y, Li ZX. Identification and behavioral assays of alarm pheromone in the vetch aphid *Megoura viciae*. J Chem Ecol. 2021:47(8-9):740–746. https://doi.org/10.1007/s10886-021-01297-4

Stappen I, Wanner J, Tabanca N, Bernier UR, Kendra PE. Blue tansy essential oil: chemical composition, repellent activity against *Aedes aegypti* and attractant activity for *Ceratitis capitata*. Nat Prod Commun. 2021:16(2):1934578X2199019–193457X21990194. https://doi.org/10.1177/1934578x21990194

Vespermann KA, Paulino BN, Barcelos MC, Pessôa MG, Pastore GM, Molina G. Biotransformation of α-and β-pinene into flavor compounds. Appl Microbiol Biotechnol. 2017:101(5):1805–1817. https://doi.org/10.1007/s00253-016-8066-7

Wajs A, Pranovich A, Reunanen M, Willför S, Holmbom B. Headspace-SPME analysis of the sapwood and heartwood of *Picea Abies*, *Pinus Sylvestris* and *Larix Decidua*. J Essent Oil Res. 2007:19(2):125–133. https://doi.org/10.1080/10412905.2007.9699244

Winston PW, Bates DH. Saturated solutions for the control of humidity in biological research. Ecology. 1960:41(1):232–237. https://doi.org/10.2307/1931961

Woodrow RJ, Grace JK, Oshiro RJ. Comparison of localized injections of spinosad and selected insecticides for the control of *Cryptotermes brevis* (Isoptera: Kalotermitidae) in naturally infested structural mesocosms. J Econ Entomol. 2006:99(4):1354–1362. https://doi.org/10.1603/0022-0493-99.4.1354

Zhao J, Wang Z, Li Z, Shi J, Meng L, Wang G, Cheng J, Du Y. Development of lady beetle attractants from floral volatiles and other semiochemicals for the biological control of aphids. J Asia-Pac Entomol. 2020:23(4):1023–1029. https://doi.org/10.1016/j.aspen.2020.08.005

Zilberman D, Lewis VR. Economic framework to assess the impact of banning pesticides, with application to sulfuryl fluoride for drywood termites (Blattodea: Kalotermitidae) in California. J Econ Entomol. 2024:117(1):1–7. https://doi.org/10.1093/jee/toad200