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The efficacy of the semiochemical repellent verbenone to reduce ambrosia beetle attack on healthy and *Ceratocystis*-infested ʻōhiʻa trees

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ABSTRACT

The *Ceratocystis* fungal disease complex, rapid ʻōhiʻa death (ROD), has killed over one million ʻōhiʻa (*Metrosideros polymorpha*), the keystone tree species of Hawaiʻi. The causal fungi can be spread by invasive ambrosia beetles (Coleoptera: Curculionidae) through fungal inoculum found on their bodies and in the frass they produce. Thus, there is a critical need to manage beetle attack on ʻōhiʻa trees to curtail subsequent pathogen spread and disease development. In this experiment at Waiākea Forest Reserve, we tested the potential of the semiochemical repellent, verbenone, in a commercial formulation, to protect healthy and ROD-affected ʻōhiʻa from ambrosia beetle attack in each of the 2022 and 2023 field seasons. Landing rates of ambrosia beetles on healthy and diseased trees were quantified over 16 weeks each year, using sticky traps on ethanol-baited trees that also received either a low (72 g) or high (108 g) dose of verbenone or untreated controls. In addition, we used gas-chromatography mass-spectrometry (GC–MS) to measure verbenone emission over 16 weeks in 2022. We found that the low dose of verbenone is the most effective and economical application to reduce beetle landing on healthy ʻōhiʻa trees and the high dose may be needed to reduce attraction to diseased ʻōhiʻa. Despite a large decrease in verbenone emission levels by week eight, the low dose effectively reduced beetle captures on healthy trees for 14 weeks and the high dose reduced landing rates on diseased trees for 10–14 weeks. Our results indicate verbenone may significantly lower ambrosia beetle attack on ʻōhiʻa, and thus, lead to reduction in the spread of ROD.

1. Introduction

Introduced forest pests and pathogens can devastate ecosystems outside of their native range, especially when keystone tree species are negatively affected (Jacobs et al., 2013; Copeland et al., 2023). In the United States, notable catastrophic diseases include chestnut blight, Dutch elm disease, laurel wilt, and more recently, rapid ʻōhiʻa death (ROD). Chestnut blight, caused by the fungus *Cryphonectria parasitica*, in combination with chestnut ink disease, caused by *Phytophthora cinnamomic*, have contributed to the disappearance of American chestnut (*Castanea dentata*) from its native range in North America in 60 years (Jacobs et al., 2013). Dutch elm disease, caused by *Ophiostoma novo-ulmi* and *Ophiostoma ulmi* and vectored by bark beetles, was introduced to the United States in the 1930s and had killed nearly half of all American elms some 40 years later (Hubbes, 1999). Laurel wilt, caused by *Harringtonia lauricola* and vectored by the ambrosia beetle, *Xyleborus glabratus* Eichhoff, has killed at least 320 million redbay trees (*Persea borbonia*) (Hughes et al., 2017a). Native Hawaiian ʻōhiʻa lehua (ʻōhiʻa; Myrtaceae: *Metrosideros polymorpha*) forests have been seriously impacted by ROD, caused by the fungal pathogens *Ceratocystis lukuohia* and *Ceratocystis huliohia*, killing at least one million trees (Keith et al., 2015; Barnes et al., 2018; Cannon et al., 2022). The loss of these

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Fig. 1. The efficacy of a commercial verbenone product was tested on ʻōhiʻa (*Metrosideros polymorpha*) trees in the field, and the emission of the active ingredient was measured over time. (A) The verbenone product was applied with a caulking gun to ʻōhiʻa trees. Trees were equipped with falcon tubes containing ethanol as an attractant and clear sticky cards for monitoring beetle capture. (B) A healthy ʻōhiʻa tree at Waiākea Forest Reserve was monitored for ambrosia beetle attack when treated with the verbenone product. (C) The verbenone product was dispensed onto plywood and weathered in the field for verbenone emission determination. (D) The plywood samples were brought back to the laboratory to measure the emission of verbenone every four weeks using gas chromatography-mass spectrometry. All photos by Kylle Roy.

keystone tree species has had devastating effects on both the environment and human-cultural dynamics (Schlarbaum et al., 1998; Roy et al., 2024a).

ʻŌhiʻa forests are vital to the stability of Hawaiian ecosystems and watersheds, and the trees themselves serve as the foundation of the forests (Gruner, 2004). Comprising 350,000 acres across the Hawaiian Islands, the majority (70 %) of ʻōhiʻa remain on the Island of Hawaiʻi (Gon et al., 2006). ʻŌhiʻa trees have a broad elevational and habitat range, growing from sea level to tree line in different habitats ranging from barren lava fields to wet forests. Nearly 500 endemic arthropods and 13 surviving avian honeycreepers depend on ʻōhiʻa for habitat and food (Gruner, 2004; Pratt et al., 2009). The leaves and plant parts of ʻōhiʻa are used in medicine, woodworking, and dance in addition to chants, songs, and stories (Malo, 1951; Abbott, 1992; Roy et al., 2024a).

Of the two fungi in the ROD disease complex, *C. lukuohia* is more virulent and widespread than *C. huliohia* (Fortini et al., 2019), although both fungi infect the functional xylem tissue of ʻōhiʻa and ultimately kill the tree (Hughes et al., 2020; Juzwik et al., 2024). The fungi can be directly vectored by four invasive ambrosia beetle species: *Xyleborinus* (*Xi.*) *saxesenii* (Ratzburg), *Xyleborus* (*X.*) *affinis* Eichhoff, *Xyleborus* (*X.*) *ferrugineus* (Fabricius), and *Xyleborus* (*X.*) *perforans* (Wollaston) (Coleoptera: Curculionidae: Xyleborini) (Roy et al., 2023a). Pathogen propagules are also common in frass produced by beetles when they bore into infected xylem tissue (Roy et al., 2020; Hughes et al., 2023). Infectious ROD-*Ceratocystis* spp. chlamydospores in frass can be transported in soil, wind, and potentially by water and feral ungulates (Harrington, 2013; Yelenik et al., 2020; Perroy et al., 2021).

To date, management of ROD has primarily focused on detecting and felling infested trees (Roy et al., 2021; Cannon et al., 2022). Verbenone delivered in the commercial SPLAT® formulation was recently reported to deter invasive ambrosia beetles from traps, including the four species known as vectors of ROD, indicating verbenone as a potential beetle behavioral control tool (Roy et al., 2023b). Verbenone was initially identified as a bark beetle anti-aggregation pheromone and was later identified as a general antagonistic bark and ambrosia beetle kairomone that is indicative of microbial deterioration of plant tissues (Frühbrodt et al., 2024a). The use of SPLAT® technology to administer the verbenone repellent is also logistically beneficial because it biodegrades within a year of application and does not require dispensers that need to be retrieved from the field (Fettig et al., 2020).

There is especially a critical need to protect ROD-affected trees from attack by ambrosia beetles to reduce the further production of frass containing viable fungal spores and limit the spread of the disease. However, the efficacy of verbenone administered in the SPLAT® Verb formulation (Specialized Pheromone and Lure Application Technology; a.i. 10 % 4,6,6-Trimethylbicyclo[3.1.1] hept-3-en-2-one) when applied directly on ʻōhiʻa trees has not been evaluated in Hawaiian forests. We evaluated the ability of the semiochemical repellent, verbenone, to deter ambrosia beetle landing on healthy and *Ceratocystis* infected ʻōhiʻa that were baited with ethanol. Ethanol simulates tree stress and is the most effective attractant for the ROD-associated beetles (refer to Roy et al. 2023b). In addition, we evaluated verbenone application dosage and emission longevity in the field.

2. Materials and methods

2.1. Verbenone treated ʻōhiʻa monitoring

Two 16-week field trials of verbenone treatment were conducted with healthy and ROD-affected ʻōhiʻa trees at Waiākea Forest Reserve (WFR), Island of Hawaiʻi (19.6092° N, 155.1226° W) from June 2022–October 2022 to April 2023–July 2023. In terms of native Hawaiian geographic districts, the WFR is in the ahupuaʻa of Waiākea in the moku of Hilo, on the mokupuni of the Island of Hawaiʻi. The study site elevation ranged from 260 to 533 m with an average rainfall of ~4420 mm/yr (Giambelluca et al., 2013). This forest is characterized by a dominant ʻōhiʻa tree canopy, high ROD mortality, and the presence of all invasive ROD-associated ambrosia beetles. For each trial, we established 16 plots with three ʻōhiʻa trees (15–30 cm in diameter at breast height (DBH)). The study trees in half of the plots had healthy green crowns while the remaining plots had trees exhibiting ROD-symptomatic crowns. There was at least 15 m between each tree, and at least 20 m between each plot. The symptomatic and healthy trees were selected based on an established ROD-mortality rating scale (Flint Hughes, U. S. Department of Agriculture Forest Service, Written comm. 2019), where full, green ʻōhiʻa canopies were considered healthy and those with finely branched canopies containing brown leaves were considered symptomatic. The presence of *C. lukuohia* and/or *C. huliohia* DNA in diseased trees was confirmed using the standard quantitative polymerase chain reaction (qPCR) diagnostic technique described by Heller and Keith (2018) followed by culturing for viable fungal propagules using a carrot-baiting technique modified from Moller and Devay (1968) and described in Roy et al. (2018).

Individual trees in both the healthy and diseased ʻōhiʻa plots received one of three treatments: (1) Ethanol lure (control), (2) Ethanol lure + 72 g verbenone, or (3) Ethanol lure + 108 g verbenone. Ethanol lures were attached to all trees on the bole, approximately 2 m from the base of the tree to simulate release from stressed trees (Kelsey and Westlind 2017), attracting and inducing ambrosia beetle attack (Rabaglia et al., 2019). Ethanol lure dispensers were composed of 50-ml Falcon™ tubes (Corning, Glendale, Arizona, USA) with a 9.5-mm [3/8-in] hole drilled through the cap and filled with 25 ml of 100 % Fisher Bioreagents™ Absolute Ethanol (200 proof; Fisher Scientific, Waltham, Massachusetts, USA). This system was designed to deliver an approximate release rate of 0.99 ± 0.09 g/day (as determined in Roy et al. 2023b). Two ethanol dispensers were attached to each tree, using 1.6-mm aluminum wire and cable ties, one on the north and the other facing the south. Both dispensers were secured at a 45° angle to the bole with a staple through the attaching cable tie to reduce rainfall inundation.

Verbenone repellents (SPLAT® Verb formulation; ISCA Technologies, Riverside, California, USA) were administered with a 750-g caulking gun equipped with a trigger stop to consistently deliver 3 g of product (ISCA Technologies, Riverside, California, USA). We tested the lowest and highest dose label rates of the verbenone repellent, using the manufacturer's custom trigger stop, at 72 g and 108 g, respectively. Each repellent treatment was administered directly onto the ʻōhiʻa bark as four equal-sized dollops (18 and 27 g dollops, respectively) in each cardinal direction at 1.3 m height, and the lures were hung just below the repellents. To determine beetle landing rates on these trees, two 12 in × 12 in clear panel traps [30.5 cm × 30.5 cm] (sticky traps; Alpha Scents, Inc., Canby, Oregon, USA), one north and the other south facing, were affixed to each tree with staples, directly below the lures and repellents (Fig. 1A, B). Sticky traps were collected every two weeks, at which time, they were replaced with new traps, and lures were replenished. All captured ambrosia beetles were identified according to Samuelson (1981), Wood (1982), and voucher specimens of each species were deposited at the B.P. Bishop Museum, Honolulu, Hawaiʻi.

2.2. Verbenone emission (mean peak area)

To estimate emission of the active ingredient over time, we applied the verbenone matrix to plywood squares and placed them in a screened field carousel cage adjacent to the study site. Four replicates of repellents were administered in 72-g dollops onto 15- x 15-cm plywood squares that were suspended from the carousel within the cage by 1.6-mm aluminum wire (Fig. 1C). Every four weeks during the 16-week field study in 2022, the treated squares were transported to the laboratory to collect released headspace volatiles. Headspace volatiles were collected using a system consisting of a custom-made 2.4 L glass chamber (Chemglass Life Sciences, Vineland, New Jersey, USA) with an inlet at the top for ambient airflow and one outlet at the bottom (both 7 mm inside diameter) connected to a vacuum that pulled air at 0.6 L/min. Volatiles were collected on a single ORBO™-32 standard charcoal tube filter (Supelco, Bellefonte, Pennsylvania, USA) that was connected to the outlet (Fig. 1D). In addition, a second ORBO™-32 standard charcoal tube filter was used to filter ambient air through the inlet port at the top of the chamber. Volatiles were collected for 15 min and eluted from the outlet ORBO fiber with 500 μl of methylene chloride (Supelco, Bellefonte, Pennsylvania, USA), and the plywood squares containing the repellent were returned to the outdoor cage after volatile collection.

Samples were immediately analyzed using gas chromatography-mass spectrometry (GC–MS). An autosampler injected 1 μl of sample into an Agilent GC–MS (Agilent Technologies, Santa Clara, California, USA; Model numbers 7890B and 5977A, respectively) equipped with a polar column (DB-WAXETR; 30-*m* × 0.25 mm × 0.25-μm film thickness; Agilent Technologies, Santa Clara, California, USA), with a flow rate of 1 ml/min and helium carrier gas under splitless mode (1 min sampling) with an inlet temperature of 250 °C. Oven temperature was initially held at 40 °C for five minutes, then ramped to 250 °C at 15 °C/min, and held at 250 °C for 5 min. The identity of verbenone was confirmed by comparing mass spectra to those in the National Institute of Standards and Technology (NIST) mass spectral library (ca. 120,000 spectra; ChemStation Version D.05.01; Hewlett Packard Corp., Palo Alto, California, USA). The mean peak area of verbenone was calculated for each sample by integrating the area under the peak in the total ion abundance chromatogram and then averaged among replicates for each collection period.

2.3. Statistical analyses

We calculated the effectiveness of verbenone to reduce ROD-associated ambrosia beetle attack according to Abbott (1925) and Frühbrodt et al. (2024b). Percent effectiveness was expressed as 100 * $(X–Y) / X$, where X = the mean number of beetles captured in the control, and Y = the mean number of beetles captured on verbenone-treated trees.

Data from field trials were analyzed using generalized linear mixed models (GLMM) that assumed a negative binomial distribution to assess the influence of repellent treatment (fixed effect) on the capture of individual beetle species and combined ROD-associated beetle counts on

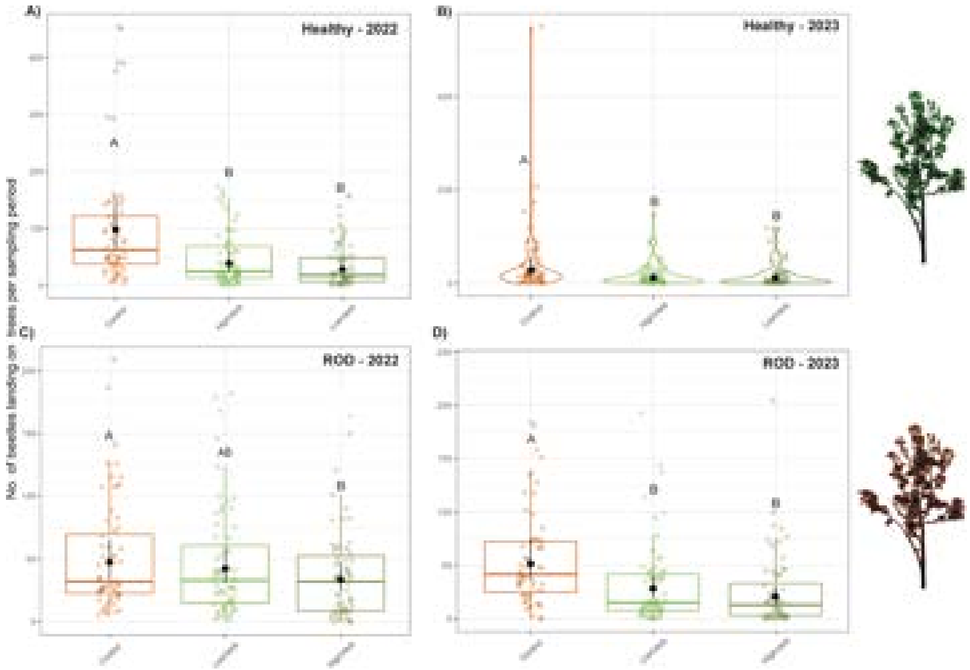

Fig. 2. Number of rapid ʻōhiʻa death (ROD)-associated ambrosia beetles (combined counts of *Xyleborinus saxesenii, Xyleborus affinis, Xyleborus ferrugineus*, and *Xyleborus perforans*) captured on (A) healthy ʻōhiʻa (*Metrosideros polymorpha*) trees in 2022 field trials and (B) 2023 field trials and (C) ROD-affected trees in 2022 and (D) 2023 at Waiākea Forest Reserve, Island of Hawaiʻi. Ethanol-baited control trees are in brown, low dose of 72 g of the commercial verbenone product with ethanol lures in lime green, and high verbenone dosage of 108 g per tree with ethanol lures is in dark green. Modeled data are black dots with 95 % confidence intervals. Refer to Tables S1, S6, S11, and S16.

sticky cards for both healthy and diseased ʻōhiʻa. We included a random effect of week to account for variation in beetle capture over time due to vagaries of weather and seasonality. A type II negative binomial distribution was applied to the beetle trap captures from healthy ʻōhiʻa and a type I negative bionomial distribution was applied for ROD-affected trees based on diagnostic plots and statistical analyses including Akaike Information Criterion (AIC) scores. Two agricultural pests that were abundant in trap capture, *Xylosandrus (Xa.) compactus* (Eichhoff) and *Xylosandrus (Xa.) crassiusculus* (Motschulsky) were included in individual species analyses. Data from each year were analyzed separately.

In addition, GLMMs assuming negative binomial distribution were used to assess the interaction effect of repellent treatment and time on the capture of all ROD-associated ambrosia beetles for each trial. Similarly, a type II negative binomial distribution was applied to the ROD-associated beetle counts for healthy ʻōhiʻa and a type I negative binomial distribution was applied for those from diseased trees.

Data management, modeling, and visualizations were done in R version 4.4.1 (R Core Team, 2022) using the tidyverse (Wickham et al., 2019), glmmTMB (Brooks et al., 2017), DHARMa (Hartig, 2022), cowplot (Wilke, 2020), emmeans (Lenth, 2023), and ggeffects (Lüdecke, 2018) packages. Statistical significance was defined as $P < 0.05$.

3. Results

The percent effectiveness of verbenone for all ROD-associated ambrosia beetles for the healthy tree field trials in 2022 was 66 % for the low dose and 54 % for the high dose. In 2023, the effectiveness was 59 % for the low dose and 58 % for the high dose verbenone. For the ROD-affected ʻōhiʻa, the percent effectiveness was 11 % for the low dose and 29 % for the high dose of verbenone in 2022 and 43 % for the low dose and 56 % for the high dose in 2023.

In the 2022 field trial, significantly lower numbers of the ROD-associated ambrosia beetle species (*Xi. saxesenii, X. affinis, X. ferrugineus,* and *X. perforans*) were captured on healthy ʻōhiʻa trees treated with a high or low dose of verbenone than those baited with ethanol alone when beetle species data were analyzed individually or when combined (Fig. 2A; Tables 1; S1-5). There were no differences in ROD-associated beetle capture between high and low-dose verbenone treatments on healthy trees.

In the 2023 field trial for healthy ʻōhiʻa trees, when data were combined for the ROD-associated beetles, both the high and low doses of verbenone treatment significantly reduced ambrosia beetle landing compared to that on ethanol-baited control trees (Fig. 2B; Table S6). When individual beetle species were analyzed separately, results were similar for *Xi. saxesenii* and *X. affinis* but not *X. ferrugineus* and *X. perforans*. Both high and low doses of verbenone reduced *Xi. saxesenii* and *X. affinis* captures on healthy ʻōhiʻa trees, relative to those baited

Table 1
The repellency of different verbenone dosages to ambrosia beetle species on ethanol-baited healthy ʻōhiʻa (*Metrosideros polymorpha*) trees at Waiākea Forest Reserve, Island of Hawaiʻi, from June 2022 to October 2022.

Species	Mean (± SE) number of beetles captured/tree/sampling period		
	Ethanol + High Dose Verbenone (108 g)	Ethanol + Low Dose Verbenone (72 g)	Ethanol Only
*Xyleborinus saxesenii**	40.30 ± 5.28^{a}	27.8 ± 4.06^{a}	77.80 ± 11.80^{b}
*Xyleborus affnis**	3.09 ± 0.69^{a}	3.48 ± 0.70^{a}	11.80 ± 1.70^{b}
*Xyleborus ferrugineus**	0.25 ± 0.08^{a}	0.20 ± 0.06^{a}	0.89 ± 0.16^{b}
*Xyleborus perforans**	2.84 ± 0.50^{a}	3.47 ± 0.82^{a}	11.20 ± 1.73^{b}
Xylosandrus compactus	0.53 ± 0.10^{a}	0.69 ± 0.12^{a}	1.95 ± 0.35^{b}
Xylosandrus crassiusculus	5.31 ± 1.52^{a}	5.02 ± 1.14^{a}	41.90 ± 5.98^{b}

$N = 8$ trees per treatment; beetles were collected every other week for 16 weeks or 8 collection periods. Means followed by different letters within species rows indicate significant differences among treatments (refer to Tables S2–5).
* = Associated with rapid ʻōhiʻa death.

Table 2
The repellency of different verbenone dosages to ambrosia beetle species on ethanol-baited healthy ʻōhiʻa (*Metrosideros polymorpha*) trees at Waiākea Forest Reserve, Island of Hawaiʻi, from April 2023 to July 2023.

Species	Mean (± SE) number of beetles captured/tree/collection		
	Ethanol + High Dose Verbenone (108 g)	Ethanol + Low Dose Verbenone (72 g)	Ethanol Only
*Xyleborinus saxesenii**	16.20 ± 3.04[a]	16.20 ± 3.16[a]	39.0 ± 9.48[b]
*Xyleborus affnis**	0.63 ± 0.16[a]	0.36 ± 0.09[a]	1.64 ± 0.30[b]
*Xyleborus ferrugineus**	0.23 ± 0.07[a]	0.41 ± 0.10[ab]	0.66 ± 0.13[b]
*Xyleborus perforans**	0.39 ± 0.09[ab]	0.22 ± 0.07[a]	0.77 ± 0.18[b]
Xylosandrus compactus	0.41± 0.09[a]	0.45 ± 0.09[a]	1.88 ± 0.32[b]
Xylosandrus crassiusculus	1.61 ± 0.26[a]	1.48 ± 0.26[a]	21.0 ± 1.98[b]

N = 8 trees per treatment; beetles were collected every other week for 16 weeks or 8 collection periods. Means followed by different letters within species rows indicate significant differences among treatments (refer to Tables S7–10).
[*] = Associated with rapid ʻōhiʻa death.

Table 3
The repellency of different verbenone dosages to ambrosia beetle species on ethanol-baited rapid ʻōhiʻa death-affected ʻōhiʻa (*Metrosideros polymorpha*) trees at Waiākea Forest Reserve, Island of Hawaiʻi, from June 2022 to October 2022.

Species	Mean (± SE) number of beetles captured/tree/collection		
	Ethanol + High Dose Verbenone (108 g)	Ethanol + Low Dose Verbenone (72 g)	Ethanol Only
*Xyleborinus saxesenii**	23.20 ± 2.77[a]	35.20 ± 4.36[b]	32.30 ± 3.97[b]
*Xyleborus affnis**	6.48 ± 1.30[a]	5.25 ± 0.97[a]	9.69 ± 1.45[b]
*Xyleborus ferrugineus**	0.75 ± 0.14[a]	0.67 ± 0.11[a]	1.11 ± 0.17[a]
*Xyleborus perforans**	5.61 ± 1.28[a]	4.45 ± 1.02[a]	8.02 ± 1.16[b]
Xylosandrus compactus	0.86 ± 0.20[a]	0.98 ± 0.19[a]	2.75 ± 0.50[b]
Xylosandrus crassiusculus	4.83 ± 1.24[a]	4.22 ± 0.86[a]	37.2 ± 4.33[b]

N = 8 trees per treatment; beetles were collected every other week for 16 weeks or 8 collection periods. Means followed by different letters within species rows indicate significant differences among treatments (refer to Tables S12–15).
[*] = Associated with rapid ʻōhiʻa death.

with ethanol alone (Tables 2; S7, S8). For *X. ferrugineus,* the high dose of verbenone also significantly reduced beetle capture on ʻōhiʻa trees, but the number of beetles captured on low dose verbenone-treated trees was intermediate between the high dose and control (Tables 2; S9). In contrast, the low dose verbenone significantly reduced the capture of *X. perforans* on healthy ʻōhiʻa trees compared to the ethanol-only controls, and capture with the high dose verbenone was intermediate (Tables 2; S10).

For the ROD-affected ʻōhiʻa in the 2022 field trial, when the ROD-associated beetle counts were combined, only the high dose of verbenone significantly reduced ambrosia beetle landing (Fig. 2C; Table S11). When species were analyzed separately, both verbenone dosages significantly reduced *X. affinis* and *X. perforans* captures on trees compared to ethanol-baited controls (Tables 3; S13, S15). There were no differences in beetle capture between high and low dose verbenone treatments for those species. The capture of *Xi. saxesenii* was significantly reduced with the high verbenone dosage compared to the low dosage and control (Tables 3; S12). Differences among *X. ferrugineus* beetle captures from both verbenone dosages approached significance compared to untreated controls (Tables 3; S14).

Table 4
The repellency of different verbenone dosages to ambrosia beetle species on ethanol-baited rapid ʻōhiʻa death-affected ʻōhiʻa (*Metrosideros polymorpha*) trees at Waiākea Forest Reserve, Island of Hawaiʻi, from April 2023 to July 2023.

Species	Mean (± SE) number of beetles captured/tree/collection		
	Ethanol + High Dose Verbenone (108 g)	Ethanol + Low Dose Verbenone (72 g)	Ethanol Only
*Xyleborinus saxesenii**	23.20 ± 4.18[a]	29.2 ± 4.52[a]	51.1 ± 5.36[b]
*Xyleborus affnis**	0.50 ± 0.11[a]	0.81 ± 0.19[a]	2.00 ± 0.29[b]
*Xyleborus ferrugineus**	0.44 ± 0.09[a]	0.73 ± 0.13[ab]	0.94 ± 0.15[b]
*Xyleborus perforans**	0.27 ± 0.09[a]	0.66 ± 0.19[a]	1.28 ± 0.27[b]
Xylosandrus compactus	0.50 ± 0.10[a]	0.50 ± 0.12[a]	1.31 ± 0.18[b]
Xylosandrus crassiusculus	1.72 ± 0.38[a]	2.00 ± 0.29[a]	27.90 ± 3.36[b]

N = 8 trees per treatment; beetles were collected every other week for 16 weeks or 8 collection periods. Means followed by different letters within species rows indicate significant differences among treatments (refer to Tables S17–20).
[*] = Associated with rapid ʻōhiʻa death.

In the 2023 field trial plots of ROD-affected ʻōhiʻa, both the high and low dose verbenone treatments significantly reduced landing rates of ROD-associated ambrosia beetles (Fig. 2D; Table S16). Similarly, when the species were analyzed separately, significantly fewer ***Xi. saxesenii, X. affinis***, and ***X. perforans*** were captured on trees treated with verbenone compared to ethanol-baited control trees, and there was no difference in beetle capture between verbenone dosages (Tables 4; S17, S18, S20). The high dosage of verbenone significantly reduced *X. ferrugineus* capture on diseased ʻōhiʻa trees compared to the ethanol-only baited trees, and the low dose of verbenone led to an intermediate reduction in *X. ferrugineus* capture (Tables 4; S19).

When analyzing ROD-associated beetle landing over time for the healthy ʻōhiʻa plots, the high verbenone treatment significantly reduced beetle capture through week 12 in both 2022 and 2023 (2022 high verbenone: $\beta = 0.67 \pm 0.20$, Z ratio $= 3.39$, $P < 0.01$; 2023 high verbenone: $\beta = 0.42 \pm 0.17$, Z ratio $= 2.52$, $P = 0.03$; Fig. 3A, B**)**, where only the low verbenone treatment was effective until week 14 in both years as well (2022 low verbenone $\beta = 0.77 \pm 0.25$, Z ratio $= 3.12$, $P < 0.01$; 2023 low verbenone $\beta = 0.57 \pm 0.21$, Z ratio $= 2.72$, $P = 0.02$; Fig. 3A, B). When analyzing ROD-associated beetle captures over time for the ROD-affected trees, the high verbenone treatment repelled beetles relative to untreated controls through week 10 in 2022 ($\beta = 0.36 \pm 0.123$, Z ratio $= 2.94$, $P < 0.01$; Fig. 3C) and through week 14 in 2023 ($\beta = 0.47 \pm 0.19$, Z ratio $= 2.43$, $P = 0.04$; Fig. 3D). Moreover, the low verbenone dosage repelled beetles relative to the ethanol controls in beetle landing through week 12 in 2023 ($\beta = 0.46 \pm 0.16$, Z ratio $= 2.89$, $P = 0.01$; Fig. 3D), but not at any timepoint in 2022 (Fig. 3C).

The level of verbenone emission declined sharply over the first eight weeks, with a more gradual decrease throughout the remainder of the study period based on GC–MS measurements. Over a period of four months, verbenone emission, relative to that at week 0, decreased by 36 % at week 4, 84 % at week 8, 93 % at week 12, and 96 % at the end of the study (week 16; Fig. 4).

Lastly, we found both dosages of verbenone were equally effective at reducing captures of the two non-ROD associated beetles, *Xa. compactus* and *Xa. crassiusculus*, on both healthy and ROD-affected ʻōhiʻa trees in both 2022 and 2023 field trials compared to non-treated controls (Tables 1–4; S21–S27).

4. Discussion

This study revealed that verbenone, administered in the SPLAT® Verb formulation, effectively reduces trap capture and thereby attacks

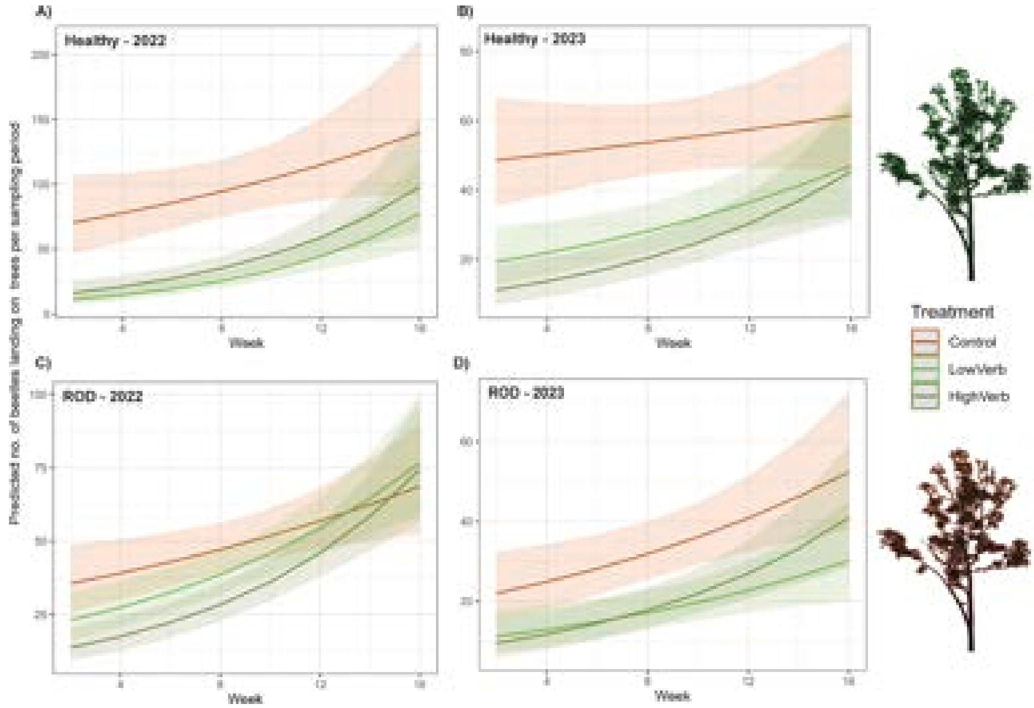

Fig. 3. Predicted number of rapid ʻōhiʻa death (ROD)-associated ambrosia beetles (combined counts of *Xyleborinus saxesenii, Xyleborus affinis, Xyleborus ferrugineus*, and *Xyleborus perforans*) captured on (A) healthy ʻōhiʻa (*Metrosideros polymorpha*) trees in 2022 field trials and (B) 2023 field trials and (C) ROD-affected trees in 2022 and (D) 2023 over time at Waiākea Forest Reserve, Island of Hawaiʻi. Ethanol-baited control treatments are in brown, low dose of 72 g of the commercial verbenone product with ethanol lures in lime green, and high verbenone dosage of 108 g per tree with ethanol lures in dark green. Shaded areas are 95 % confidence intervals.

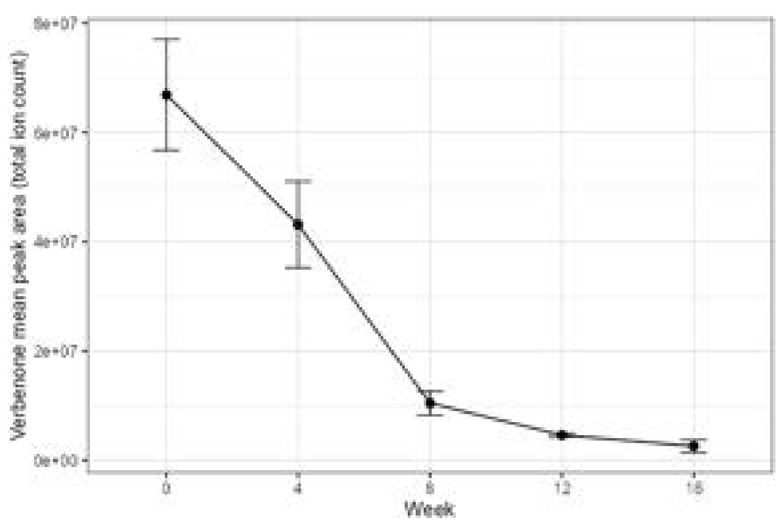

Fig. 4. The emission (mean peak area) of verbenone, administered in the commercial verbenone formulation, over time at Waiākea Forest Reserve, Island of Hawaiʻi, from June 2022 to October 2022. Verbenone emission was measured using gas chromatography-mass spectrometry and expressed as total ion count.

by all four of the invasive ROD-associated ambrosia beetles on both healthy and ROD-affected ʻōhiʻa. Despite the rapid initial decrease in emissions of the active ingredient within the first eight weeks of deployment (Fig. 4), the low dosage of 72 g of verbenone is sufficient to deter landings on healthy ʻōhiʻa for at least 14 weeks (Fig. 3A, B), while the high dosage of 108 g is effective at reducing beetle landings on diseased ʻōhiʻa for 10–14 weeks (Fig. 3C, D).

In two years of testing verbenone on healthy ʻōhiʻa trees, both dosages of the repellent were equally effective against all ROD-associated ambrosia beetles when combined, with some differences for individual species noted (Figs. 2A-B, 3A, B; Tables 1 and 2). As of 2024, the low dose of verbenone costs about $13 USD to treat an individual tree whereas cost for the high dose is $20 USD. Therefore, the use of the low-dose verbenone may be both sufficient and economical for deterring beetle attack and possible subsequent ROD-*Ceratocystis* infection of healthy ʻōhiʻa trees. According to our models over time, the longevity of the product may not last a full 16 weeks, and re-application may be necessary 14 weeks post initial application.

The high dosage of verbenone consistently reduced captures of ROD-associated ambrosia beetle species on ROD-affected ʻōhiʻa trees when data were combined, with some inconsistencies within individual species and years, although the data were not as clear as those of healthy trees (Figs. 2C-D; 3C-D; Tables 3 and 4). The application of verbenone on ROD-affected ʻōhiʻa trees would likely reduce ambrosia beetle attack and therefore subsequent frass production, decreasing the amount of inoculum available in the environment. It is also likely that ROD-*Ceratocystis*

Fig. 5. Potential rapid ʻōhiʻa death (ROD) containment strategy where new ROD outbreaks are occurring. The brown tree(s) represent ROD-affected ʻōhiʻa (*Metrosideros polymorpha*) trees that would be treated with the high dose (108 g) of verbenone and the surrounding green trees are healthy ʻōhiʻa treated with the low dose (72 g).

spp. diseased trees are emitting ethanol stress signals (Roy et al., 2023a) as well as other plant and fruity kairomones common to *Ceratocystis* species (Mailula et al., 2020). These volatiles may attract ambrosia beetles, especially *X. ferrugineus* and *X. affinis,* that are known vectors in other related *Ceratocystis*-tree pathosystems (Saunders and Knoke, 1967; Herrera and Grillo, 1989; Romero et al., 2022). An increase in attractive plant and fungus volatile emissions may explain why, compared to the healthy ʻōhiʻa, the difference in repellent effect of verbenone on diseased ʻōhiʻa data was less clear and longevity of the product was reduced. Therefore, based on our data and the potential presence of other kairomones that may compete with the repellent effect of verbenone, the high dosage may be necessary to treat diseased ʻōhiʻa, with re-treatment at 10–14 weeks.

In Hawaiʻi, peak ambrosia beetle abundance occurs from May to September, similar to that in the contiguous United States, (Dr. Curtis Ewing, CAL FIRE, oral communication, 2023). It is noteworthy that in 2023 our trial was conducted earlier (April–July) in the peak activity period of these beetles compared to the 2022 trial (June–October), which may explain the generally lower beetle captures in the second year and slight variabilty of verbenone treatment effectiveness on individual beetle species. Additionally, there may be major differences in beetle community assemblages at sites and various elevations (Roy et al., 2020; 2023b), so it is important to consider potential effects on endemic beetle populations, especially at high elevations. Of note, despite significant beetle reduction, beetle attacks were not completely prevented by verbenone treatment. *Ceratocystis* can be a deadly tree killer (Ploetz et al., 2013), and a single contaminated beetle attacking a healthy tree could result in tree death. Therefore, investigation into other repellents such as those containing methyl salicylate and biological control agents may be warranted.

Verbenone is an effective repellent for ambrosia beetles in other insect pest-tree species systems (Burbano et al., 2012; VanDerLaan and Ginzel, 2013; Hughes et al., 2017b; Martini et al., 2020; Rivera et al., 2020; Tobin and Ginzel, 2023). However, Rivera et al. (2020) determined the effective space of SPLAT® Verb, when deployed in an avocado orchard in Florida, was less than one meter. Considering that ʻōhiʻa trees are typically canopy trees, reaching heights of 10 m or more, and the ROD-associated beetles colonize at different bole heights of ROD-diseased ʻōhiʻa (Roy et al., 2020), additional applications higher into the canopy may be necessary. For beetles such as *X. ferrugineus*, that colonize the lower 3.6 m of ʻōhiʻa boles, however, a single breast-height treatment may be sufficient. This beetle also produces copious amounts of frass, and therefore greater levels of ROD-*Ceratocystis* spp. inoculum, compared to other beetle species (Roy et al., 2020).

Verbenone, administered in the SPLAT® Verb formulation, is biodegradable and holds much potential for effective suppression of ROD-associated ambrosia beetle attack, both to prevent healthy ʻōhiʻa from being inoculated by beetle vectors and to suppress further beetle colonization of diseased trees, subsequent frass production, and disease spread. Particularly in new ROD-outbreak areas, verbenone could serve as a containment tool, with newly detected ROD trees receiving the high dose treatment while surrounding healthy trees are treated with the low dose formulation (Fig. 5). Considering the peak ambrosia beetle season from May to September in Hawaiʼi, a single application of verbenone to healthy ʻōhiʻa trees once a year may be sufficient to protect trees and managers may choose to re-apply the treatment to ROD-affected trees mid-season (as longevity varied from year to year) to ultimately reduce frass inoculum in the environment. Furthermore, verbenone could also be a viable alternative to felling of ROD-affected trees during summer pupping season months, when the endangered Hawaiian hoary bat (ʻōpeʻapeʻa; *Lasiurus semotus*) have non-volant young at tree roosts including in ʻōhiʻa (Montoya-Aiona et al., 2023). Further research would be beneficial to understand the potential of verbenone beetle repellent formulations to prevent the spread of ROD on a landscape scale. The data support that verbenone is an effective tool for limiting the spread of ROD and supporting conservation management of ʻōhiʻa forests.

CRediT authorship contribution statement

Kylle Roy: Writing – review & editing, Writing – original draft,

Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Dan Mikros:** Writing – review & editing, Validation, Methodology, Data curation. **Dong H. Cha:** Writing – review & editing, Software, Resources, Methodology, Funding acquisition. **Ellen J. Dunkle:** Writing – review & editing, Methodology, Data curation. **Jennifer Juzwik:** Writing – review & editing, Resources, Methodology. **Matthew Ginzel:** Writing – review & editing, Methodology, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tfp.2024.100735.

Data availability

Data are available at ScienceBase:https://doi.org/10.5066/P1XZVBPW

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