Technical Report HCSU-099

EFFECTIVENESS OF RAPID ʻŌHIʻA DEATH MANAGEMENT STRATEGIES AT A FOCAL DISEASE OUTBREAK ON HAWAIʻI ISLAND

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ABSTRACT

The ongoing spread of rapid ʻōhiʻa death (ROD) in the Hawaiian Islands threatens the long-term sustainability of ʻōhiʻa lehua (*Metrosideros polymorpha*) forests throughout the state. First identified in the Puna district of Hawaiʻi Island in 2014, the disease caused by the novel fungi *Ceratocystis lukuohia* and *Ceratocystis huliohia* has now spread island-wide and was recently detected on Kauaʻi, Oʻahu, and Maui. The leading hypothesis for the spread of ROD is through airborne ambrosia beetle frass particles that contain viable *Ceratocystis* propagules, thus management efforts focus on containing this frass. At the time of this study (2017–2018), the Waipunalei site was the northernmost outbreak of ROD on Hawaiʻi Island. The focal nature of the outbreak and accessibility of the location provided the opportunity to monitor the effectiveness of two types of proposed management methods to reduce the airborne spread of potentially infective ambrosia beetle frass: tree felling and insecticide treatments. We placed 23 passive environmental samplers (PES), which monitored for airborne frass and wood particles containing *C. lukuohia* and *C. huliohia* in a grid that spanned the outbreak area over 22 weeks. Cross-vane panel traps with 1:1 methanol:ethanol lures were attached to nine of the PES to document wood-boring ambrosia and cerambycid beetle populations during the latter three months of the study. Monitoring with PES began three weeks before management and continued for one month after the last infected trees were felled. Glass microscope slides from the 23 PES were examined for airborne ambrosia beetle frass and wood particles by microscopy. DNA was extracted from the slides and tested by qPCR (quantitative polymerase chain reaction) for *C. lukuohia* and *C. huliohia*. We also investigated the correlation of beetle gallery counts with tree height and tested the efficacy of Bifen I/T insecticide (active ingredient: bifenthrin 7.9%) for preventing beetle attacks on the cut surface of ʻōhiʻa bolts (tree stem sections). Beetle trapping data revealed that the area supports a diverse community of wood-boring beetles, some of which likely attack ʻōhiʻa and may facilitate the spread of ROD. The number of beetle galleries on felled ʻōhiʻa trees decreased linearly as tree height increased. We also observed significantly fewer beetle attacks on Bifen I/T treated ʻōhiʻa bolts than non-treated bolts, but gallery formation nearly ceased in both treated and control bolts by week three. *Ceratocystis lukuohia* DNA was detected twenty-six times and *C. huliohia* was detected five times in the PES throughout this study. DNA detections were correlated to frass and wood counts, and the number of felled trees were correlated to wood particle counts but not frass counts. Both the timing and distribution of detections across the sampling grid indicate that tree felling may have reduced airborne detections of *Ceratocystis* DNA soon after tree felling was completed. A subsequent increase in detections after tree felling ceased may indicate that incomplete removal of infected trees and the appearance of new infections in previously asymptomatic trees could have allowed airborne detections of potentially infectious fungal propagules to once again increase.

INTRODUCTION

ʻŌhiʻa lehua (*Metrosideros polymorpha*) is the most common native tree throughout the Hawaiian archipelago and covers approximately 350,000 ha, the majority of which is on Hawaiʻi Island (250,000 ha; Gon *et al.* 2006). ʻŌhiʻa are the first trees to colonize lava flows (Smathers and Mueller-Dombois 1974). They substantially contribute to Hawaiian watersheds and provide critical habitat for the native and endangered flora and fauna of Hawaiʻi (Muller-Dombois *et al*. 2013). Additionally, the trees are extremely important to the native Hawaiian culture, which is

evidenced by the countless chants and songs (Emerson 2005, Mueller-Dombois *et al*. 2013) mentioning the tree, the many uses of the wood for weapons, statues, and building material, and the use of the flower and leaves in lei and offerings (Abbott 1992, Emerson 2005, Mueller-Dombois *et al.* 2013).

Rapid ʻōhiʻa death (ROD) is a singular name for two different pathogens that cause similar wilting of the ʻōhiʻa crown, *Ceratocystis lukuohia* and *Ceratocystis huliohia* (Keith *et al.* 2015, Barnes *et al.* 2018). The disease caused by *C. lukuohia* is referred to as Ceratocystis wilt of ʻōhiʻa and is the more virulent of the two species, whereas *C. huliohia* is associated with tree cankers, although both fungi appear to be fatal (Hughes *et al.* 2020). In addition to being more virulent, *C. lukuohia* has been detected in significantly more samples than *C. huliohia* on Hawaiʻi Island (Fortini *et al*. 2019) and is therefore considered to be a higher risk to native ecosystems. Both *Ceratocystis* fungi colonize the sapwood of ʻōhiʻa, clogging water transport. The pathogens are contained under the bark of the tree and therefore depend upon wounding to spread.

Although the epidemiology of ROD is not fully understood, current research supports the hypothesis that ambrosia beetle (Coleoptera: Curculionidae: Scolytinae) frass transported in the wind, soil, or water may be associated with disease transmission (Heller and Keith 2018, Roy *et al*. 2019, Roy *et al.* 2020b). Direct transmission associated with ambrosia beetles that attack stressed or injured trees, wounding by feral ungulates, and human activity may also contribute to the spread of the disease.

Management strategies have been developed in an attempt to slow the spread of ROD (David Benitez, National Park Service, written communication, 2018; Figure 1). These are based largely on the unproven hypothesis that the movement of infective beetle frass is a primary pathway for the spread of the diseases. As a part of these management strategies, trees that test positive for *Ceratocystis* DNA by quantitative polymerase chain reaction (qPCR) and display visible ambrosia beetle presence (frass, boring holes) are felled and covered with tarps. The tarping treatment is intended to limit access to beetles, contain sawdust and frass, increase wood drying rates, and reduce fungal survival. Due to the forested settings of many ROD infections, it is not always feasible to fell and tarp all trees. For example, felling of a ROD tree close to healthy trees may cause wounds that are susceptible to infection, or numerous infected trees in a stand may be impractical to fell and tarp. The use of insecticide as a post-felling treatment instead of tarping or as an alternative to felling trees is currently being explored.

In 2017, the Department of Land and Natural Resources Hawaiʻi State Division of Forestry and Wildlife (DOFAW) discovered a northern outbreak at Waipunalei on the Hāmākua coast between Kaʻawaliʻi and Laupāhoehoe streams. This area is considered a critical management area due to its proximity to sensitive forest habitat at Manowaialeʻe Forest and Laupāhoehoe Natural Area Reserves. Over six months from December 2017–May 2018 DOFAW and the Big Island Invasive Species Committee's (BIISC) Early Detection and Rapid Response crew collected samples from symptomatic trees to be tested and diagnosed by qPCR at U.S. Department of Agriculture Agricultural Research Services Daniel K. Inouye Pacific Basin Agricultural Research Center (USDA-ARS-DKI-PBARC).

Infected and symptomatic trees that could be felled without injuring healthy trees were cut. Felled trees in the "core area", or what was hypothesized to be the epicenter of the disease outbreak at this site, were bulldozed into larger piles. These felled trees were then sprayed with insecticide to prevent ambrosia beetle attacks.

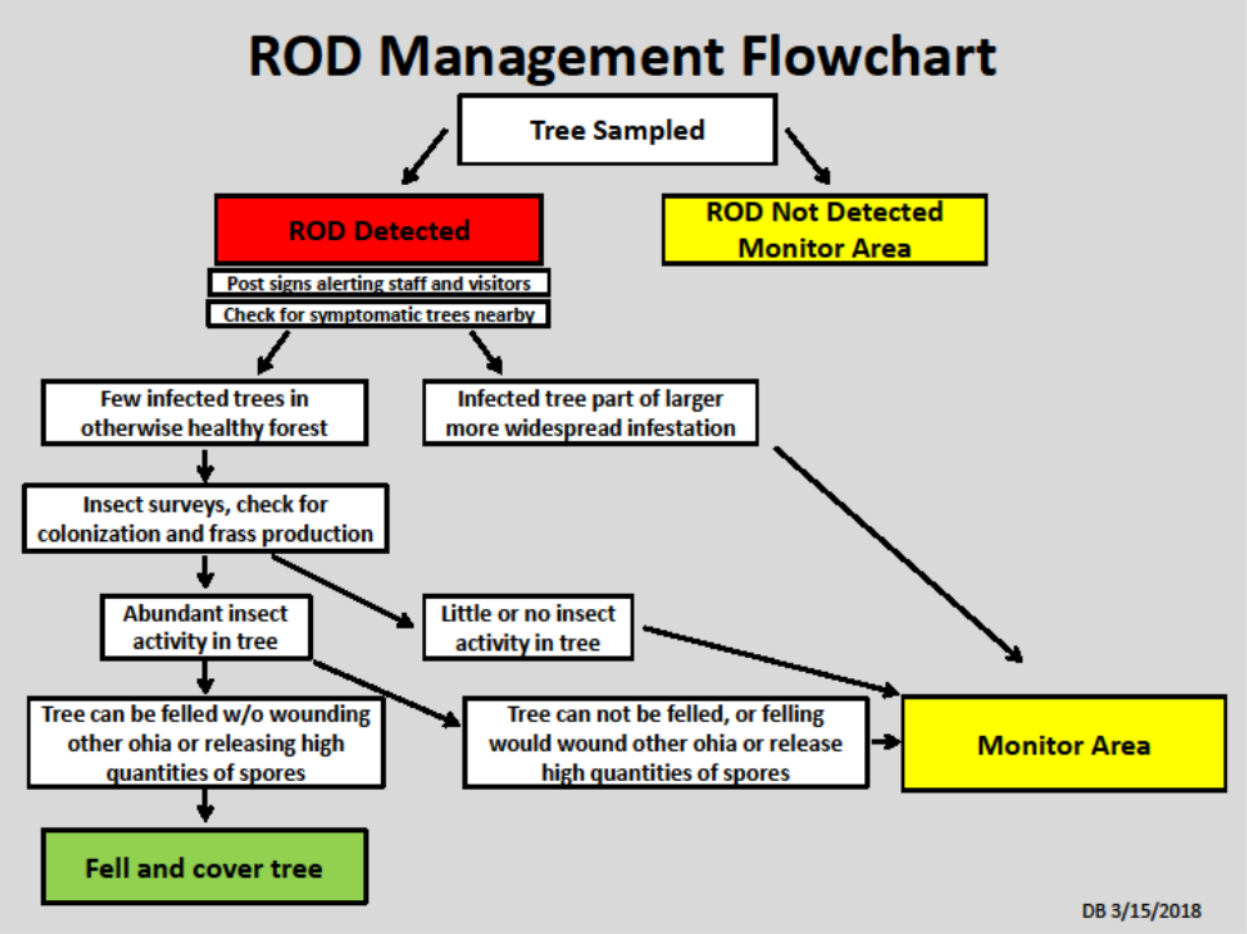

Figure 1. Rapid ʻōhiʻa death (ROD) management decision tree, developed by National Park Service ecologist David Benitez, for managers and homeowners to determine ROD-related management actions.

Before, during, and after the course of these management practices, we used passive environmental samplers (PES; Atkinson *et al.* 2019) to monitor airborne beetle frass and wood particles as well as *C. lukuohia* and *C. huliohia* DNA. We also sampled the wood-boring beetle community of the Waipunalei area with cross-vane panel traps (CVPT), estimated ambrosia beetle gallery counts on recently felled trees, and tested the efficacy of insecticide to prevent ambrosia beetle gallery formation. We hypothesized that (1) the majority of *Ceratocystis* DNA detections from PES would be in the core area, (2) management activities would decrease the amount of airborne *Ceratocystis* DNA detections in PES over time, (3) beetle frass and wood particles observed in PES would decrease after felling activities ceased, (4) ambrosia beetles associated with ROD would be present at Waipunalei with higher numbers of galleries in lower portions of trees, and (5) insecticide treatment would reduce ambrosia beetle attacks on ʻōhiʻa.

METHODS

Study Area

The experiment was conducted at an area known as Waipunalei, located on the Hāmākua Coast of Hawai'i Island (Figure 2). The management and monitoring area encompassed a 600 x 500 m rectangular section of a property situated on an elevation gradient of 460–610 m. The area was characterized by extremely degraded forest with a scattered 'ōhi'a canopy, strawberry guava (*Psidium cattleyanum*) understory, mixed patches of open pasture, and the presence of feral cattle and pigs.

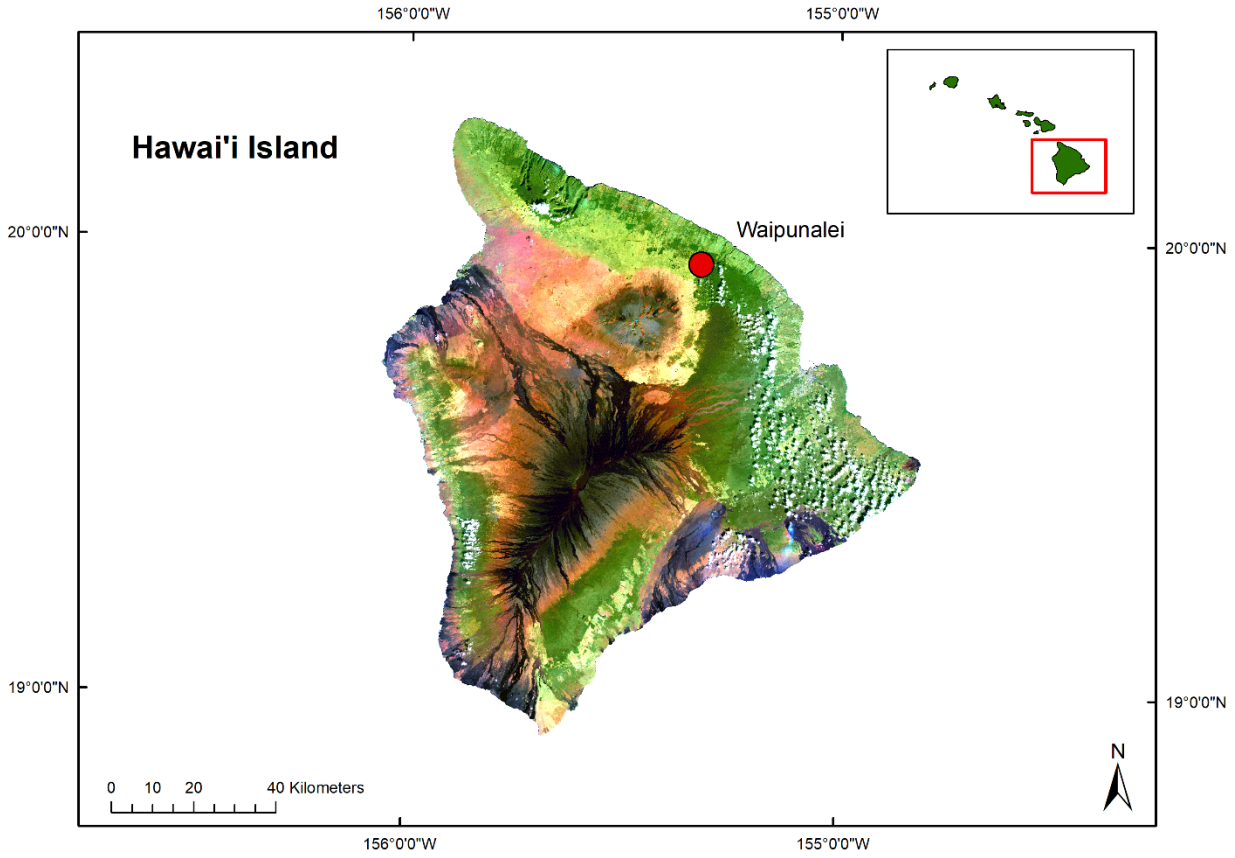

Figure 2. The study site, Waipunalei, is located on the northeastern side of Hawai'i Island. Inset map depicts the location of Hawai'i Island within the Hawaiian island chain. Base layer from Kimmet 2016.

The upper areas of the study site contained sparse patches of common ironwood (*Casuarina equisetifolia*). Lower Waipunalei, directly below the study site, contained remains of an old coffee farm and a large eucalyptus plantation. The disease outbreak was focused within a 150-meter-diameter circular area, hereafter referred to as the core area. The core area was located

at the northern, lower end of the study site while the upper area had relatively few symptomatic trees.

Management Activity

Management activities at Waipunalei took place in several overlapping phases. During the first three weeks of the study (November 14–December 4, 2017), trees infected with *Ceratocystis* were identified in the core area based on symptomology and diagnostic qPCR tests. Based on characteristic ROD symptoms, the study area was divided into two management areas: the lower, northern core area of the ROD outbreak referred to as the "treatment" area and an adjacent southern "control" area. The treatment area contained the core area including 155 symptomatic ROD trees that were felled over time, and the majority of the trees (141/155) were felled between weeks 4 and 10 (December 4, 2017–February 6, 2018; Figure 3). The control area contained 4 symptomatic trees that were felled, only one of which was confirmed for *C. lukuohia* DNA. *Ceratocystis huliohia* was detected in a few trees up slope of the control area. During weeks 15 and 16 of the study (February 20–March 6, 2018), downed trees in the core area were bulldozed into piles and limited additional tree felling took place in locations outside of the core area. The piles of downed trees in the core area were sprayed at the label rate with insecticide/termiticide (Bifen I/T [active ingredient: bifenthrin 7.9%], Control Solutions, Inc., Pasadena, Texas, USA) during weeks 20 and 21 of the study (March 27–April 10, 2018).

Passive Environmental Samplers and Cross-Vane Panel Traps

To assess the presence of airborne wood particles and ambrosia beetle frass that might contain *C. lukuohia* or *C. huliohia* propagules, 23 PES were placed as a grid throughout the study site (Figure 4). Sampler locations were initially plotted in ArcGIS v. 10.7.1, then adjusted after ground-truthing, with 11 PES in the treatment area and 12 PES in the control area. Passive environmental samplers were constructed of ductwork attached to a sheet metal vane affixed to a three-meter pole (Figure 5). The samplers can rotate freely to face the prevailing wind and collect airborne particulates on four glass microscope slides positioned at a 45° angle. Slides were covered with a piece of Scotch™ tape and smeared with a light coating of silicone grease (Beckman Coulter, Berea, California, USA) to make them sticky (Atkinson *et al.* 2019). Slides were collected and replaced weekly from November 21, 2017–April 17, 2018, during a three-week pre-management period, an intensive nine-week period where trees were felled in the core area of the outbreak, and a ten-week post-management monitoring period in which all felled trees and slash near the core area was bulldozed into piles and sprayed with insecticide.

Cross-vane panel traps (CVPT; Forestry Distributing, Boulder, Colorado, USA) baited with 100 ml of a 1:1 ethanol:methanol mixture in clear semiochemical release sleeves (Synergy Semiochemicals, British Columbia, Canada) were attached to nine PES poles (10 ft, ¾ inch electrical conduit) with a right angle, 18-inch length of ½ inch PVC pipe (Figure 5) to assess the abundance of the wood-boring beetle community in the area. Collection cups were constructed with 946 ml plastic storage containers (Ziploc® Twist 'n Loc®, SC Johnson, Racine, Wisconsin, USA) with two squares (2.5 cm^2) removed at the top and replaced with a mesh screen to allow overflow water from heavy rainfall to drain. Containers were filled with 200 ml of LowTox® propylene-glycol antifreeze (Prestone®, Danbury, Connecticut, USA) for preserving beetle catch. Traps were deployed from January 3–April 24, 2018. Antifreeze containing beetles and other insects was collected weekly from each trap by pouring any excess liquid (rainwater) through one of the screened squares at the top of the container, transferring the remaining contents

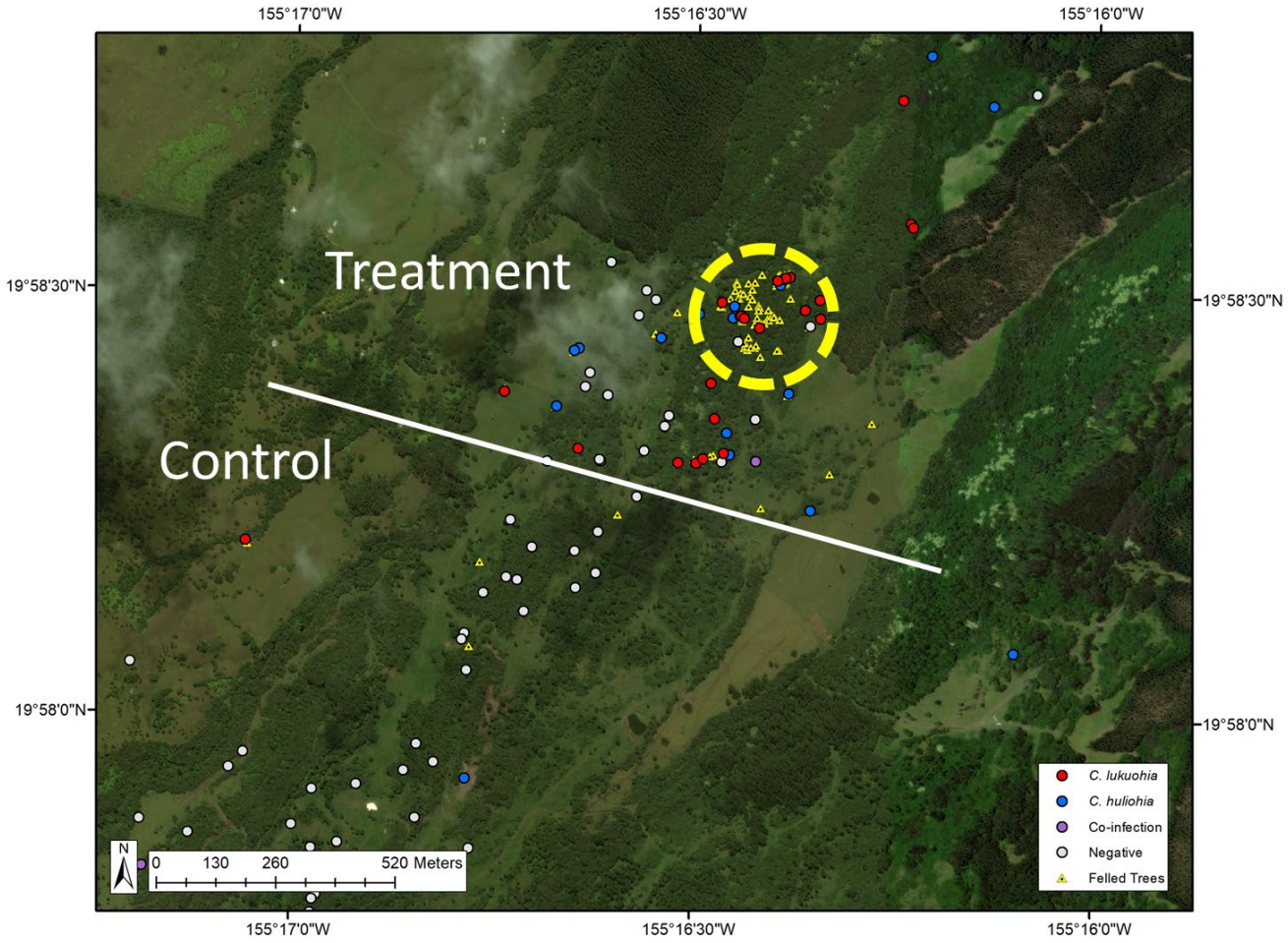

Figure 3. Diagnostic results for dead ʻōhiʻa trees sampled at the Waipunalei study site based on synthesized data from BIISC (Big Island Invasive Species Committee) and USDA-ARS-DKI-PBARC (U.S. Department of Agriculture, Agricultural Research Services Daniel K. Inouye Pacific Basin Agricultural Research Center). Felled trees are indicated by yellow triangles. Not all trees were tested for *Ceratocystis* DNA before felling. The broken yellow circle represents the core area where most management activities took place. The white line demarcates treatment and control areas. Not all of the felled trees in the control area were recorded. Base layer from Kimmet 2016.

into plastic snap-top bags, and then refilling the containers with fresh antifreeze. Collections were stored at room temperature until further processing. Beetles were identified according to Samuelson (1981) using a Leica MZ12 dissecting microscope (Leica Microsystems, Wetzlar, Germany).

DNA Extraction and Diagnostic Testing for *Ceratocystis*

After collection from PES, two-inch tape strips on each of four glass slides were cut into six

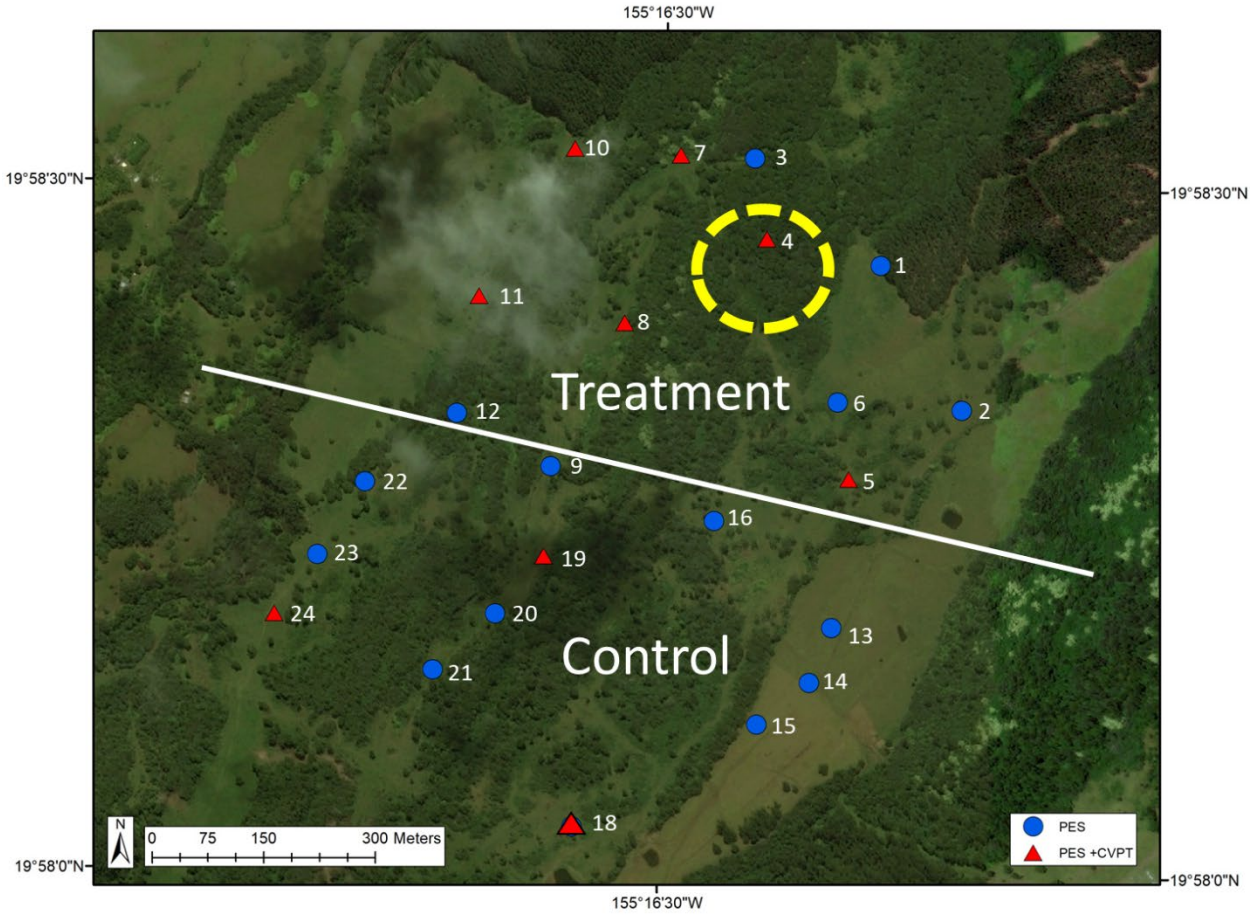

Figure 4. Map of Waipunalei management area depicting passive environmental samplers (PES) and cross-vane panel traps (CVPT). Red triangles represent the nine PES with attached CVPT. Blue circles represent PES without CVPT. PES numbers are labeled in white. The broken yellow circle represents the core area where most management activities took place. The white line demarcates treatment and control areas. Base layer from Kimmet 2016.

pieces with a sterile scalpel blade, peeled from slides, and then transferred to 2-ml screw-cap tubes containing 0.3 g of 100 µm silica beads and 0.3 g of 800 µm zirconium beads (OPS Diagnostics, Lebanon, New Jersey, USA). DNA was extracted from each of the four tape strips using the QIAmp DNA Investigator Kit (Qiagen Inc., Germantown, Maryland, USA) according to the manufacturer protocol with an initial homogenization at 4.5 m/sec for 40 seconds on a FastPrep-24™ 5G homogenizer (MP Biomedicals, Santa Ana, California, USA). Extracts from each slide were tested by qPCR for the presence of *C. lukuohia* and *C. huliohia* DNA. Positive control probes and primers were incorporated into the qPCR diagnostic reactions that amplified the presence of airborne pollen from ʻōhiʻa and other closely related species of Myrtaceae (Heller and Keith 2018). Samples were run in triplicates in 96-well plates and tested for both *C. lukuohia* and *C. huliohia* DNA on a CFX96 Real-Time System (BioRad Laboratories, Inc., Hercules, California, USA) with ultrapure water non-template controls and gBlock®

Figure 5. Passive environmental sampler (PES) with attached cross-vane panel trap (CVPT) and debarked ʻōhiʻa bole with marked ambrosia beetle gallery entrances (circles). (A) A PES and attached CVPT were affixed to metal fence posts. (B) The PES contains a wooden box with four greased microscope slides for trapping airborne particulates. (C) Entrances to ambrosia beetle galleries are circled with a black permanent marker.

oligonucleotide (Integrated DNA Technologies, Coralville, Iowa, USA) positive controls. The gBlock® consisted of a synthetic, 250-basepair region of the cerato-platanin gene qPCR target. Tape strip extractions were tested individually (i.e., four strips/extractions per trap), and a positive detection for any of the four strips was denoted as a positive detection for the entire trap.

To increase PCR sensitivity and the number of DNA detections, we retested samples with a nested qPCR procedure that first amplified a 334-bp region of the *C. lukuohia* and *C. huliohia* cerato-platanin gene with a pair of conserved forward and reverse primers (60F, 5′- TGG GCC TCT CAC TAA TAG TCT CC – 3′; 393R, 5′- CGT TGT CGA CAC GGC CAG – 3′). For this analysis, we pooled DNA extractions from each tape strip within a PES per week collection for the preamplification step (3 µl from each tape extraction for a total of 12 µl of template for each PCR reaction). Preamplification reactions were run in 25 µl volumes with 2 mM $MgCl_2$, 0.2 mM of each dNTP (deoxyribonucleotide triphosphate), 4 mM of each primer, and 1.25 units of Taq polymerase (AmpliTtaq Gold, ThermoFisher Scientific, Waltham, Massachusetts, USA). The template was amplified for 25 cycles (95°C for 30 sec, 50°C for 30 sec, 72°C each for 30 sec) with a final 10-min extension step at 72°C. After preamplification, 5 µl of the product was tested in duplicate by qPCR as described previously using the cerato-platanin primers and probes designed by Heller and Keith (2018). Positive qPCR detections from the initial qPCR analysis and the follow-up nested qPCR procedure were pooled for analysis.

Frass and Wood Particle Counts

Beginning at week 7, glass slides collected from PES were examined under a Leica MZ 200 dissecting microscope before DNA extraction for the presence or absence of ambrosia beetle frass or wood chip particles. Frass was defined as rectangular-shaped, 500-µm or smaller woody particles, and wood chips were woody particles larger than 500 µm. The presence or absence of frass and wood particles was recorded for each slide.

Wind Speed, Direction, and Rainfall

Weather data (wind speed and direction) were collected with a HOBO microstation supporting a Davis® S-WCF-M003 wind speed and direction sensor (Onset Computer Corporation, Bourne, Massachusetts, USA). The microstation and sensor were mounted on a 3-m long, ¾-inch electrical conduit that was attached to a metal fence post adjacent to PES 2 (Figures 4, 5). Wind speed, wind direction, and rainfall data were also obtained from the U.S. Forest Service weather tower at the Laupāhoehoe Unit of the Hawai'i Experimental Tropical Forest located approximately 2 miles away for comparison (Hawai'i Permanent Plot Network 2018). Wind speed and direction data were highly correlated by linear regression between the two data sets (wind speed $\beta = 0.66$, $R^2 = 0.84$, $P < 0.01$; wind direction $\beta = 0.78$, $R^2 = 0.66$, $P < 0.01$), therefore data from the U.S. Forest Service weather tower were used because they were more complete. Measurements of wind speed (m/sec) and rainfall (mm) were collected at 10-min intervals and grouped by date into the 22 sampling weeks beginning at 12:00 PM on the day that slides were installed in PES and ending at 11:50 AM on the day that slides were removed from PES. Maximum, minimum, and median wind speed (m/sec) and rainfall (mm) were calculated for each sampling week using the 10-min sampling periods.

Beetle Gallery Entrance Counts

Nine 'ōhi'a trees in the core area were felled by personnel from BIISC and examined to determine the vertical distribution of beetle gallery entrances. Six trees were examined for ambrosia beetle galleries on December 15, 2017, two to three days after felling. An additional three trees were examined for ambrosia beetle galleries on January 19, 2018, two to three days after felling. We divided the main stem into 1-meter long sections, beginning 1 meter from the base of the tree and continuing until the top of the bole was reached. The circumference of each 1-meter section was measured at the midpoint of each section. Two, 0.25-m^2 square quadrats constructed with nylon cord were used to count beetle gallery entrances in every even-numbered meter section of the tree bole. Quadrats were placed randomly within the

sections, and bark within that area was removed using a drawknife (Timber Tuff Tools, Miltona, Minnesota, USA) for improved gallery observation. Gallery entrances were marked and counted with a Sharpie® permanent marker (Figure 5C). Counts from each of the two quadrats were combined and averaged for analysis.

Insecticide Treatment

Bifen I/T, hereafter referred to as bifenthrin, was the selected insecticide to be tested by BIISC based on availability and literature. To evaluate whether this treatment reduced ambrosia beetle attacks, eight different ʻōhiʻa trees were cut into bolts one week after felling. Per each tree, four 50-cm long bolts were removed, two from the base (mean diameter 25.31 ± 1.72 cm) and two from the mid-section (mean diameter 41.19 ± 2.93 cm) along with the height of the tree. One bolt from the base and middle of each tree were paired into treatment and control groups (Figure 6). Drill shavings were collected from each bolt before treatment with bifenthrin and tested for the presence of *C. lukuohia* and *C. huliohia* DNA by qPCR (Heller and Keith 2018). After treated logs were sprayed with insecticide, new beetle gallery formation on the cut surface of each treated and untreated control bolt were counted and recorded each week over five weeks from April 2–May 1, 2018.

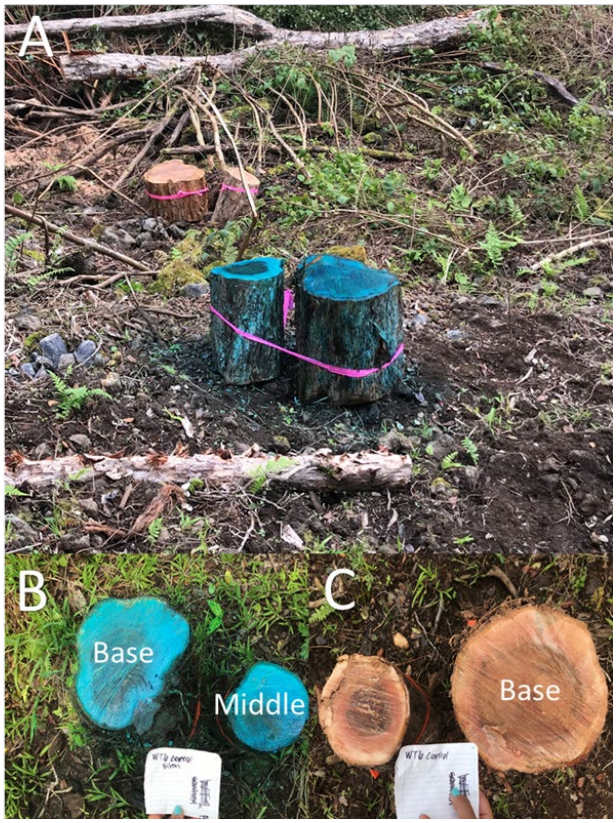

Figure 6. Assessment of bifenthrin insecticide sprays to deter ambrosia beetle boring. The number of ambrosia beetle attacks on the cut surface of ʻōhiʻa bolts was evaluated for five weeks after treatment with bifenthrin. (A) Two bolts were taken from the mid-section and base of each felled ʻōhiʻa tree and (B) either treated with bifenthrin with a blue dye incorporated or (C) left with no treatment (control).

Statistical Analysis

Statistical analyses were performed in R studio version 4.0 (R Core Team 2020). To analyze the PES data, we performed a two-way chi-square test for independence for site (control vs. treatment), activity (felling vs. monitoring), and *Ceratocystis* detections in PES. Two-sample Kolmogorov-Smirnov tests were done to compare the cumulative distribution of the number of felled trees to *Ceratocystis* DNA detections, frass particle counts, and wood particle counts, where $P < 0.05$ are not significantly correlated (Marsaglia *et al.* 2003). Simple linear regression was used to determine the correlation between wind speed and wind direction data collected from the wind sensor installed at the Waipunalei study site and U.S. Forest Service weather tower. The effect of wind speed, wind direction, and rainfall on ʻōhiʻa/Myrtaceae detections in PES was evaluated with a binomial generalized linear mixed-effects model (GLMM) accounting for the 23 PES as a random effect using the lme4 package (Bates *et al*. 2015). The best model was chosen using the AICcmodavg package and reported below (Mazerolle 2019). The best model was visualized using the effects package (Fox and Weisberg 2018).

Mean beetle entrance counts were compared to tree height by linear regression. For the insecticide trial, a paired t-test was used to compare beetle attacks in untreated and treated bolts, and statistical significance was defined as $P < 0.05$. Sample means with ± standard error estimates are reported below.

RESULTS

PES Detections of *Ceratocystis*, Wood Particles, and Frass

Positive *Ceratocystis* DNA detections by qPCR collected in PES were independent of sites (treatment vs. control) and management period (felling vs. monitoring; Fisher's exact test, $P = 0.67$; Table 1, Figure 7). *Ceratocystis* DNA was detected 26 times in PES (5.8% of 443 collections) during the study. Twenty-one of these detections consisted of *C. lukuohia* alone, while 5 (1.1% of 443 collections) consisted of both *C. lukuohia* and *C. huliohia* combined (Table 2, Figure 7). Frass and wood particles were visually detected on microscope slides collected in PES throughout the tree felling and post-felling monitoring phases of the project, with a peak at week 15 that corresponded to bulldozing and tree felling activity (Table 2).

Table 1. Contingency table of positive *Ceratocystis* DNA detections by qPCR (quantitative polymerase chain reaction) in passive environmental samplers (PES) for treatment vs. control areas and felling vs. monitoring management periods. Positive PES detections, site, and activity (felling or monitoring) were independent of each other (Fisher's exact test, $P = 0.67$).

	Control area	Treatment area	Total
Felling	4	4	8
Monitoring	6	10	16
Total	10	14	24

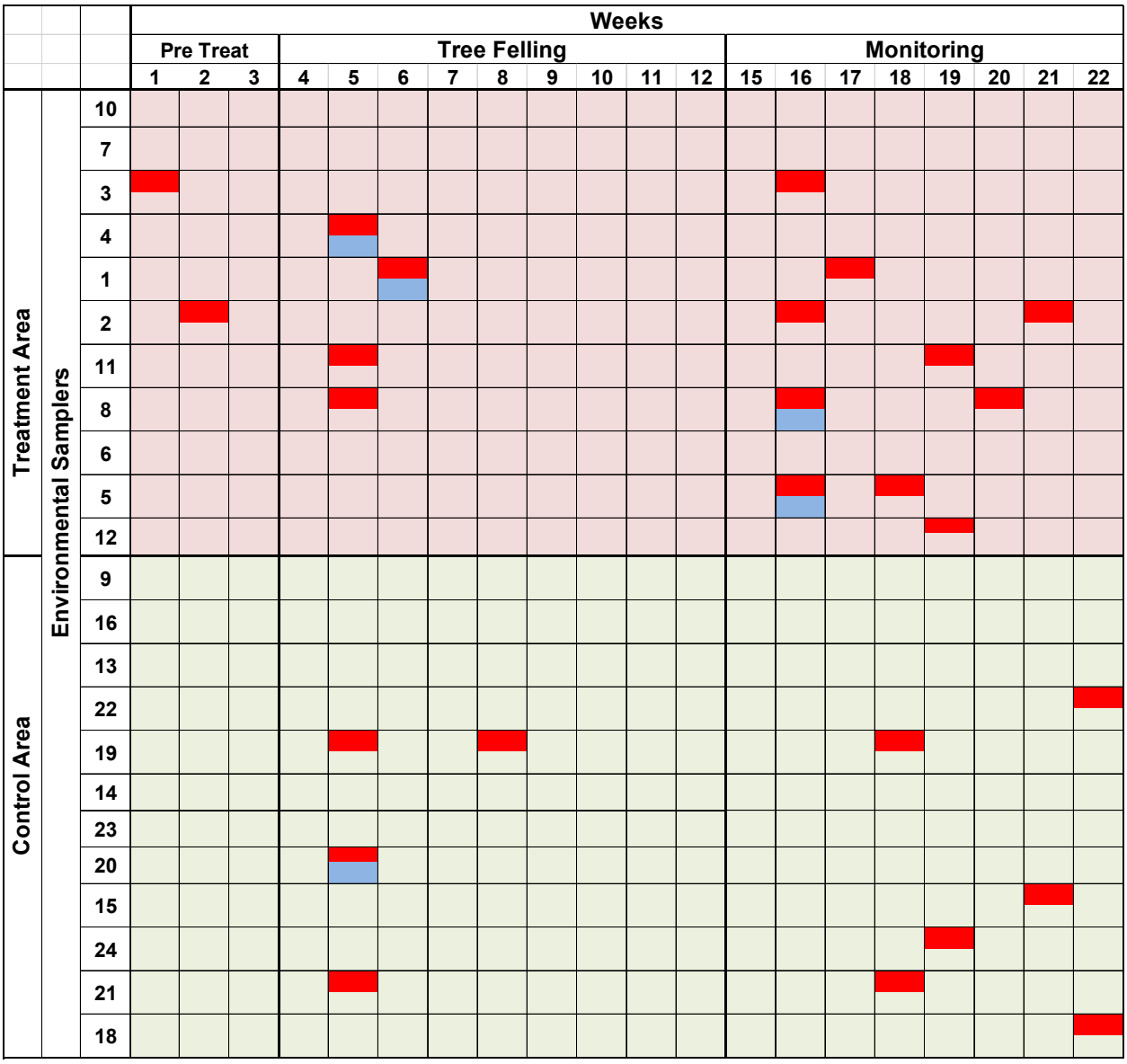

Figure 7. Schematic representation of *Ceratocystis lukuohia* and *Ceratocystis huliohia* DNA detections in passive environmental samplers (PES) based on treatment and control areas during pre-treatment, tree felling, and post-management monitoring periods. Passive environmental samplers were labeled 1–24, not including 17. Red rectangles indicate *C. lukuohia* DNA detections; blue rectangles indicate *C. huliohia* DNA detections. Dual detections from the same PES are indicated by red and blue squares. Data from weeks 13 and 14 are missing.

Because we hypothesized that the felling of infected trees over time would lead to increased visual wood particle detections and a decrease in the number of *Ceratocystis* DNA and frass visual detections, we compared the cumulative distributions of these variables using a two-sample Kolmogorov-Smirnov test (Figure 8). The cumulative distribution of *Ceratocystis* DNA detections was correlated with frass (K-S test, D = 0.38, P = 0.14) and wood particle detections

Table 2. The number of *Ceratocystis* DNA detections and frass and wood particle visual counts from the 23 passive environmental samplers, the number of trees felled, and the management actions during the study between 2017 and 2018.

Collection date	Week number	*C. lukuohia* detections	*C. huliohia* detections	Combined *Ceratocystis* detections	Traps with frass particles	Traps with wood particles	Number of trees felled	Management
Nov 14–21	1	1	0	1	NA	NA	0	-
Nov 21–29	2	1	0	1	NA	NA	0	-
Nov 29–Dec 4	3	0	0	0	NA	NA	0	-
Dec 4–13	4	0	0	0	NA	NA	31	Felling
Dec 13–19	5	6	2	6	NA	NA	50	Felling
Dec 19–26	6	1	1	1	NA	NA	13	Felling
Dec 26–Jan 3	7	0	0	0	6	0	0	-
Jan 3–9	8	1	0	1	4	9	0	-
Jan 9–16	9	0	0	0	3	0	2	Felling
Jan 16–24	10	0	0	0	12	2	47	Felling
Jan 24–30	11	0	0	0	7	2	0	Felling
Jan 30–Feb 6	12	0	0	0	8	1	8	Felling
Feb 6–13	13	NA	NA	NA	10	1	0	-
Feb 13–20	14	NA	NA	NA	8	4	0	-
Feb 20–28	15	0	0	0	15	22	6	Bulldozing/felling
Feb 28–Mar 6	16	4	2	4	8	13	2	Bulldozing/felling
Mar 6–12	17	1	0	1	5	1	0	-
Mar 12–19	18	3	0	3	9	1	0	-
Mar 19–27	19	3	0	3	10	3	0	-
Mar 27–Apr 2	20	1	0	1	11	2	0	Bifenthrin
Apr 2–10	21	2	0	2	0	0	0	Bifenthrin
Apr 10–17	22	2	0	2	9	1	0	-
Total	-	26	5	26	125	62	159	-

We were unable to obtain qPCR (quantitative polymerase chain reaction) results for weeks 13 and 14 (NA). Frass was defined as 500-µm or smaller woody particles, and wood particles were woody particles larger than 500 µm; particle counts did not begin until week 7 (NA). Tree felling, bulldozing, and insecticide treatments were done in the core area of the study site. Pre-management monitoring was conducted during weeks 1–3; tree felling was focused on weeks 4–12; post-management monitoring with limited tree felling, bulldozing, and insecticide treatments were done during weeks 13–21.

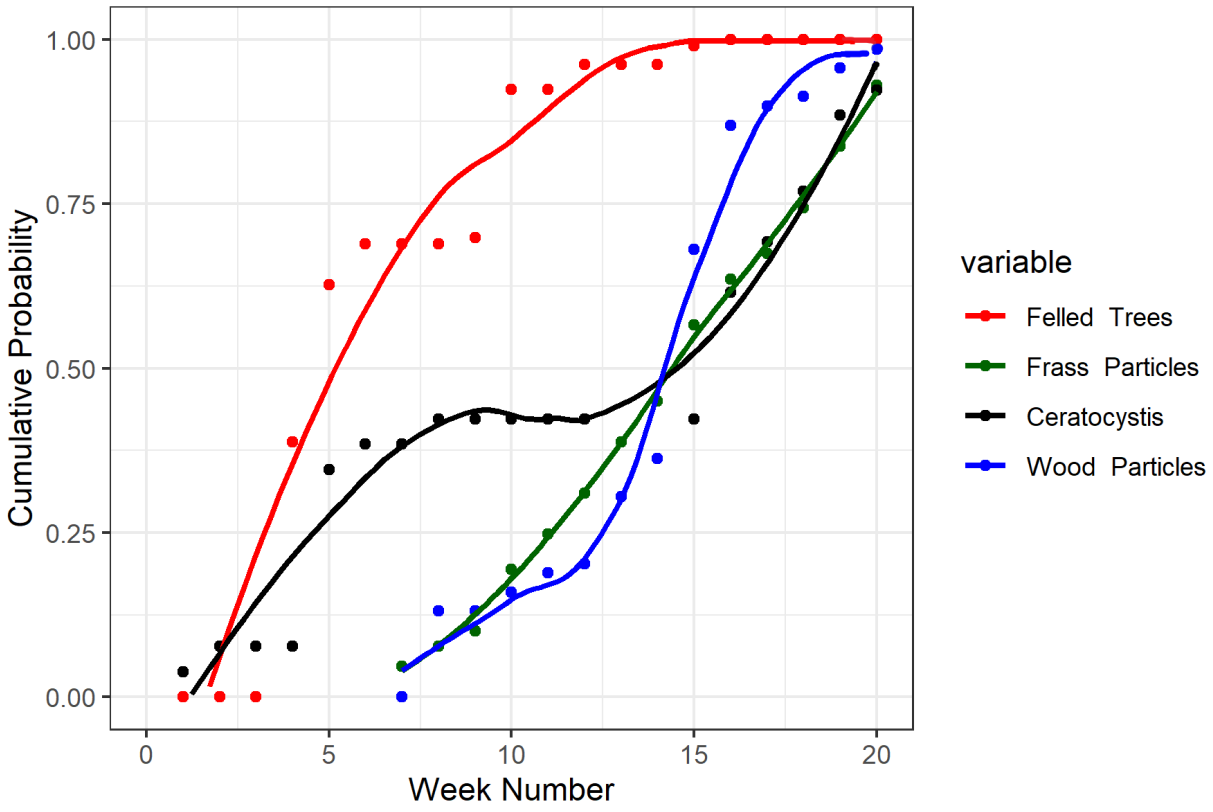

Figure 8. The cumulative probability of tree felling, frass particle visual detections, *Ceratocystis* DNA detections, and wood particle visual detections over time.

(K-S test, D = 0.39, P = 0.12), and the cumulative distribution of felled trees and wood particles were correlated (K-S test, D = 0.36, P = 0.17). However, the cumulative distribution of felled trees and frass particles (K-S test, D = 0.46, P = 0.04) as well as felled trees and *Ceratocystis* DNA detections (K-S test, D = 64, P < 0.01) were not correlated. The cumulative curves for both frass and *Ceratocystis* detections differed substantially in shape. While cumulative frass counts increased at a relatively constant rate throughout the study (Table 2, Figure 8), cumulative *Ceratocystis* detections reached a plateau by week 8 as tree felling neared completion, remained flat between weeks 9–15, and then began to increase again at a relatively constant rate at week 16.

Effect of Wind Speed, Direction, and Rainfall on PES Detections

The total number of *Ceratocystis* DNA detections were too small for analysis of wind speed, wind direction, and rainfall effects by GLMM. However, frequent detections of ʻōhiʻa/Myrtaceae DNA in PES allowed the use of these data as proxies for the potential movement of airborne *Ceratocystis* propagules. The best GLMM fit by maximum likelihood (Laplace approximation) combined maximum weekly wind speed and maximum weekly rainfall (Max windspeed β ± SE = 0.86 ± 0.11, z = 7.45, P < 0.01; Maximum rainfall β ± SE = -0.21 ± 0.04, z = 0.04, P < 0.01). The probability of detection increased by over 200% with every 1 m/sec increase in wind speed. In contrast, the probability of ʻōhiʻa detection decreased by 81% for every millimeter of rain (Figure 9).

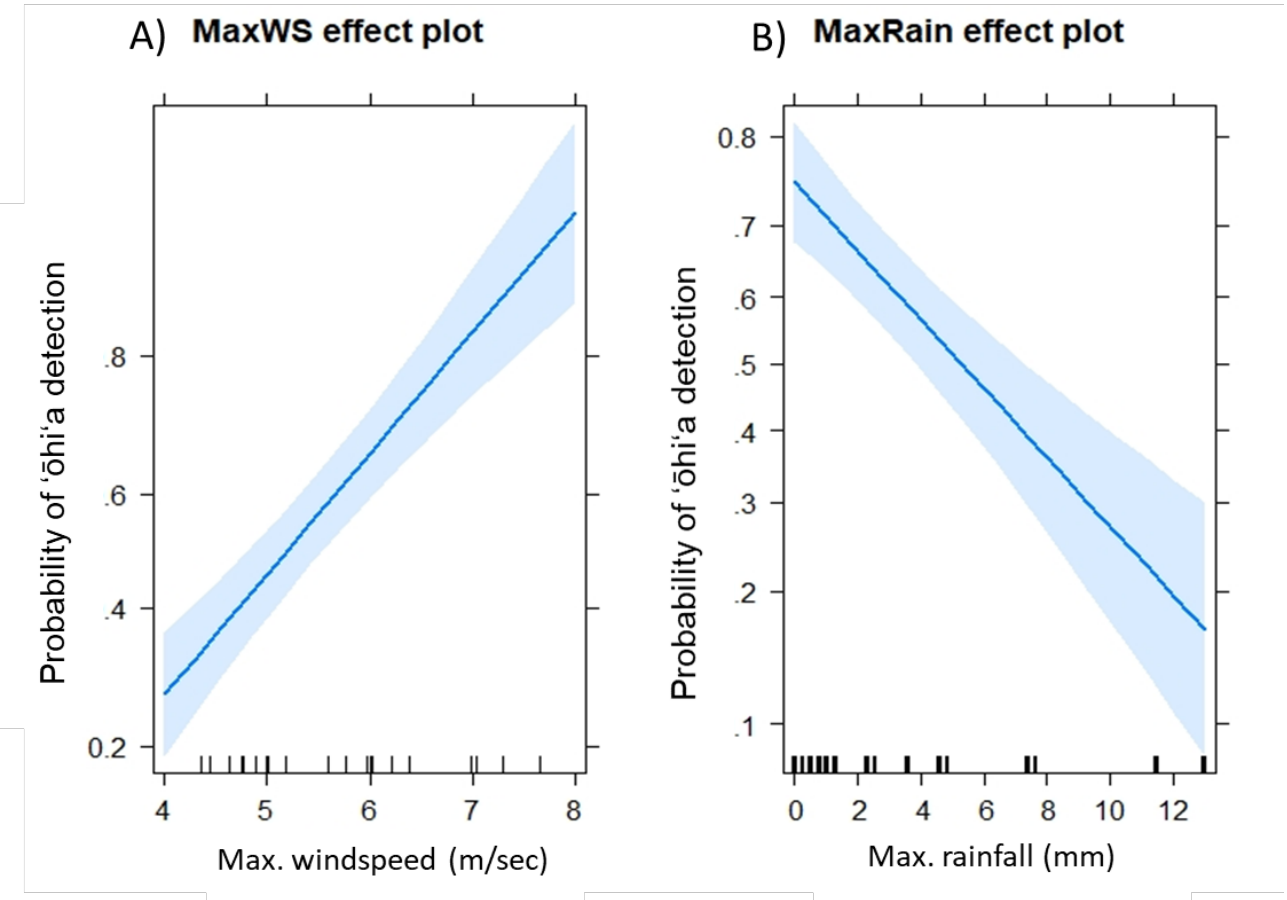

Figure 9. The probability of ʻōhiʻa DNA detection in the passive environmental sampler (PES) traps set up in Waipunalei, Hawaiʻi. Effects of (A) mean maximum wind speed (m/sec; MaxWS) and (B) mean maximum rainfall (mm; MaxRain) on the probability of detecting ʻōhiʻa DNA via qPCR (quantitative polymerase chain reaction) analysis; ± 95% confidence intervals are indicated by blue shading. Probability was determined by a binomial generalized linear mixed-effects model (ʻōhiʻa detection ~ MaxWS + MaxRain (1| PES)).

Cross-Vane Panel Traps

A total of 2,045 wood-boring beetles were captured in CVPT during Jan 23–Apr 24, 2018, including seven species of ambrosia beetles (Table 3). Three of the seven species have previously been reared from ROD-infected ʻōhiʻa including *Xyleborinus saxesenii*, *Xyleborus affinis*, and *Xyleborus ferrugineus* (Curtis Ewing, University of Hawaiʻi, Mānoa, oral communication, 2018; Roy *et al.* 2020b). Overall, *X. saxesenii* was the most abundant beetle collected, followed by *Hypothenemus* spp. (collectively two or more species). *Xyleborus affinis* and *X. saxesenii* were collected at all trap locations, and *X. ferrugineus* was collected at nearly all trap locations, excluding traps 23 and 5 (Figure 4).

Beetle Gallery Entrance Counts

We found beetle galleries along the entire length of ʻōhiʻa boles (main stems) to diameters as small as 3.4 cm. The number of beetle galleries decreased linearly as tree height increased in our linear regression analysis (β = -0.47, R^2 = 0.31, $P > 0.01$; Figure 10).

Table 3. Counts and relative abundance (percent) of wood-boring beetles collected in cross-vane panel traps at Waipunalei with 1:1 EtOH:MeOH lure over 13 weeks. Included are ambrosia beetles (subfamily Scolytinae, tribe Xyleborini), bark beetles (subfamily Scolytinae, tribe Cryphalini), and longhorn beetles (Cerambycidae).

	Week number														
Species	11	12	13	14	15	16	17	18	19	20	21	22	23	Total counts	Abundance (%)
Xyleborinus saxesenii	6	190	25	120	40	22	0	189	25	186	114	4	36	957	47
Xyleborus affinis	2	6	3	1	2	1	0	10	9	16	2	2	12	66	3
Xyleborus ferrugineus	1	10	0	2	1	1	0	3	1	7	4	2	0	32	2
Xylosandrus compactus	2	15	2	6	2	4	1	22	0	14	14	5	5	92	4
Xylosandrus crassiusculus	17	34	28	44	14	13	0	50	37	75	39	9	19	379	19
Xylosandrus germanus	0	1	1	2	1	0	0	0	1	4	0	1	1	12	<1
Xylosandrus morigerus	1	0	0	1	3	0	0	0	0	0	0	0	0	5	<1
Hypothenemus spp.	44	99	9	43	6	7	1	118	8	41	86	2	12	476	23
Cerambycid spp.	4	5	0	8	2	2	0	0	0	1	2	2	0	26	1

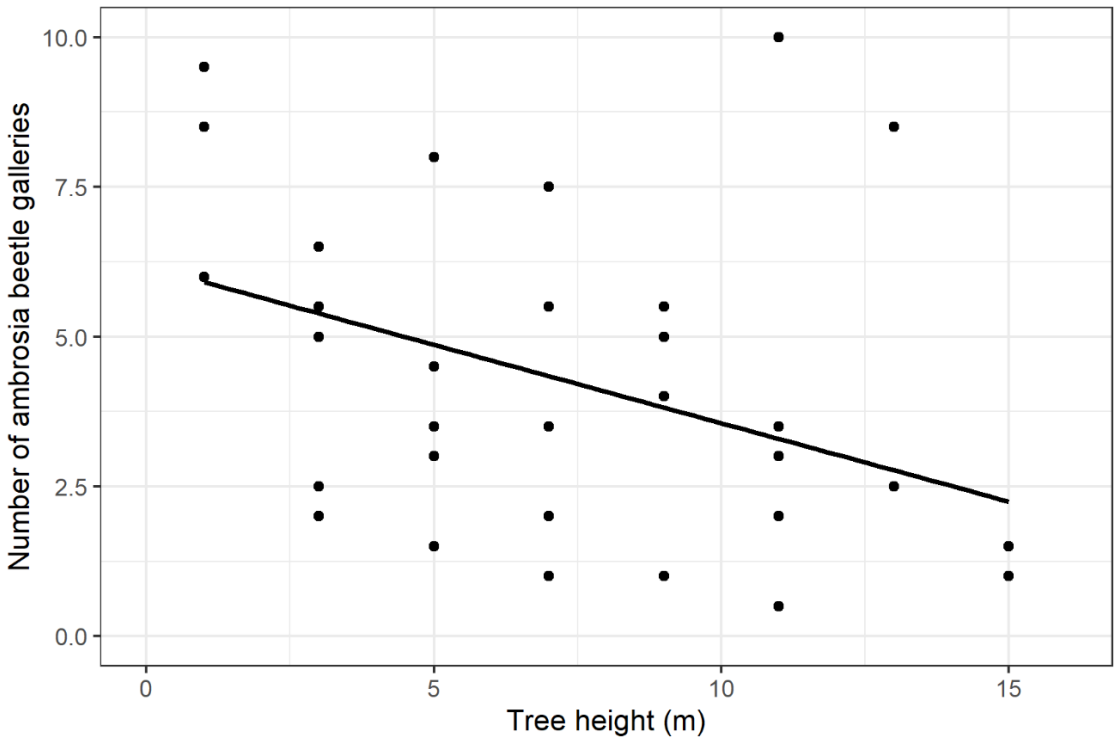

Figure 10. The relation between ʻōhiʻa tree height (m) and ambrosia beetle galleries as sampled with counts within two, 0.25 m^2 quadrats per stem section (β = -0.47, R^2 = 0.31, $P > 0.01$).

Evaluation of Insecticide Treatment

Bifenthrin-treated cut surfaces of bolts were attacked by ambrosia beetles significantly fewer times than non-treated bolts (t = -2.67, df = 7.00, $P = 0.03$; Table 4). New beetle gallery attacks were consistently observed on untreated bolt surfaces more often than bifenthrin-treated bolts during the first three weeks of evaluation, however, on weeks 4 and 5 a single new beetle attack occurred on both treatments (Figure 11).

Table 4. Total new ambrosia beetle attacks on the cut surface of ʻōhiʻa bolts treated with bifenthrin compared to untreated controls (t = -2.67, df = 7.00, $P = 0.03$).

Tree number	Number of beetle attacks on bifenthrin-treated ʻōhiʻa	Number of beetle attacks on untreated ʻōhiʻa
WT1	4	6
WT2	4	7
WT3	0	0
WT4	4	7
WT5	0	3
WT6	1	7
WT7	11	9
WT8	0	7
Total	24	46

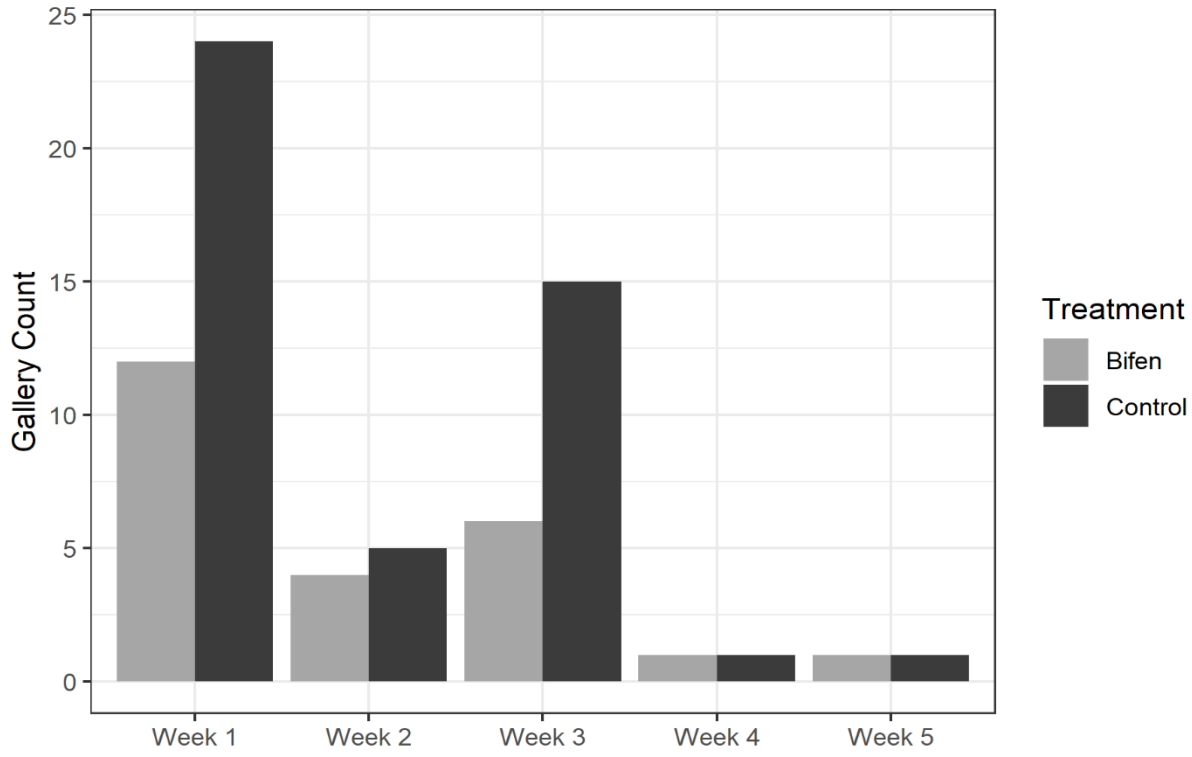

Figure 11. The total number of beetle attacks on untreated and bifenthrin-treated bolts during the five-week study period. Bolts treated with bifenthrin (Bifen I/T) were attacked less frequently than control bolts during the first three weeks of the experiment, although by weeks 4 and 5 beetle gallery formation was reduced to one gallery on both treatments.

DISCUSSION

Effect of Management Activities on Detections of *Ceratocystis* DNA, Wood Particles, and Frass

While the isolated, focal nature and easy accessibility of the outbreak at Waipunalei provided an opportunity to monitor the effectiveness of tree felling to decrease the spread of ROD, it was impossible to conduct a fully replicated experiment due to patchy land ownership, landowner access restrictions, and the absence of comparable nearby outbreaks. Instead, we used an adjacent, untreated control area on the same parcel of land where the prevalence of symptomatic trees was lower than the core area for weekly comparisons of airborne frass, wood particles, and *Ceratocystis* DNA. We were particularly interested in whether the detections of *Ceratocystis* DNA were independent of time (felling vs. monitoring periods) and location (treatment vs. control area). We hypothesized that felling infected trees would remove inoculum from the air column and reduce airborne detections of frass and *Ceratocystis* DNA. Therefore, we expected cumulative detection curves for frass and *Ceratocystis* DNA to be inversely related, i.e., as the number of felled trees increased throughout the study period, new detections of *Ceratocystis* DNA and frass particles would decline, and cumulative detections would reach a plateau. We also hypothesized that wind speed, rainfall, and wind direction may play a role in airborne dispersal of potentially infective beetle frass. While evaluating the change

in *Ceratocystis*-infected trees over time is the most direct way to measure the efficacy of control measures, we used an environmental monitoring approach with the hope that the effects of management could be measured more closely in real-time by documenting changes in abundance of potentially infective airborne frass.

We used a combination of qPCR and visual inspection of sticky slides to document the presence of *Ceratocystis* DNA, beetle frass, and wood particles that were collected from a grid of 23 PES dispersed over treatment and control areas. While both *C. lukuohia* and *C. huliohia* DNA were detected, only 5.8% (26/443) of collections from PES were positive for *Ceratocystis* DNA. This low detection rate may be related to the sensitivity of the qPCR assay that was designed to amplify the single-copy nuclear cerato-platanin gene (Heller and Keith 2018), the low abundance of *Ceratocystis* DNA in airborne frass, or some combination of the two. In a previous study conducted in the lower Puna District of Hawai'i Island, the rate of *Ceratocystis* DNA detections was as high as 15%, although PES at that site were placed much closer to infected trees compared to the random deployment of samplers in this study (Atkinson *et al*. 2019).

Luchi *et al*. (2013) found that a qPCR assay designed to multicopy internal transcribed spacer (ITS) genes of *Ceratocystis platani* was approximately 25 times more sensitive than assays based on the cerato-platanin gene and warned that using a cerato-platanin qPCR diagnostic for environmental samples risks underestimating the quantity of airborne inoculum. The very high cycle threshold values that we observed from initial tests of our samples and the often random, stochastic nature of those detections indicate that the qPCR assay we used was at the limit of detectability for *C. lukuohia* and *C. huliohia* DNA in our environmental samples. To increase DNA detections using the available qPCR assay, we used a nested qPCR protocol to improve sensitivity by first amplifying a larger fragment of the cerato-platanin gene from higher template volumes and then testing products from those reactions by qPCR (Takahashi *et al*. 2008). By using this approach, we were able to double the number of *Ceratocystis* detections. The number of positive detections were still too low for statistical evaluation by complex models.

By contrast, detections of 'ōhi'a/Myrtaceae in PES were abundant during the study, validating that the PES were picking up substantial numbers of airborne particulates likely including 'ōhi'a pollen, seeds, flower parts, leaf trichomes, and wood particles. Both maximum wind speed and rainfall had significant effects on 'ōhi'a/Myrtaceae detections in the PES. The probability of detection increased by over 200% for every 1 m/sec increase in wind speed and decreased by 80% for every millimeter of rainfall recorded during weekly sampling periods. Declines in detections as rainfall increased were most likely related to the removal of particulates from the air column. It is likely *Ceratocystis* DNA detections follow similar patterns, but our DNA detection assays were not sensitive enough to discern this.

As might be expected, airborne detections of wood particles in PES were significantly correlated with tree cutting, but tree cutting had no significant effect on the abundance of airborne frass or number of *Ceratocystis* detections in samplers based on cumulative detection curve comparisons. Similarly, tests of independence between location (treatment vs. control areas) and time (treatment vs. monitoring periods) were not significant, indicating that neither location nor management activity had significant effects on cumulative detections of airborne *Ceratocystis* DNA. These findings may be expected given the scale of the outbreak (hundreds of infected trees) and the likelihood that the outbreak was well established rather than incipient by the time management actions began. Given that there is a time lag between infection and the appearance of symptoms, it is likely that many infected trees were not yet visibly symptomatic

during the tree felling phase of the study and therefore not felled despite potentially producing viable *Ceratocystis* inoculum in the form of beetle frass. Incomplete removal of infected trees and new tree infections may have masked declines in airborne *Ceratocystis* DNA associated with tree felling.

We did, however, observe a distinct plateau or flattening of cumulative *Ceratocystis* DNA detections during weeks 9–16 (Figures 7, 8). This plateau was concurrent with the completion of most tree felling. Interestingly, there were no declines in airborne frass detections during this period, which might indicate that not all infected ʻōhiʻa were felled or that not all frass contained *Ceratocystis* as demonstrated in Roy *et al.* 2019. Environmental frass detections may also be produced from other tree species including strawberry guava, eucalyptus, and paperbark trees on site. While there was no statistically significant evidence of decreased *Ceratocystis* DNA detections, the flattened curve indicates that tree felling reduced the abundance of potentially infective frass. This effect was short-lived as potentially new infections and shifts of asymptomatic to symptomatic trees could have led to the production of frass containing *Ceratocystis* DNA again after week 16.

Ambrosia Beetle Gallery Formation and Abundance at Waipunalei

Given the key role that ambrosia beetles play in the release of frass from trees infected with ROD and the absence of detailed information about their vertical distribution, we documented the overall ambrosia beetle community at the study site with CVPT and also examined gallery distribution along the boles of recently felled trees. Before this study, it was generally believed by the ROD community that the ambrosia beetle species associated with ROD only attacked the lower sections of ʻōhiʻa trees. Because beetle frass from the top portions of trees may be more likely to spread by the wind column, we wanted to understand what portions of the trees were more heavily colonized by beetles. Because felling and tarping trees are often not practical due to tree size, the number of infected trees, or their location adjacent to healthy trees, this information is critically important for determining whether the use of insecticides can be an alternative treatment and whether felling is likely to be beneficial.

We found that beetle gallery numbers were negatively correlated to tree height, with fewer numbers in the small-diameter canopy branches. However, there were still a considerable number of galleries well above what could be reached with insecticide using a manual backpack sprayer, and insecticide application on standing trees may have little or no effect on overall beetle survival and frass production (Roy *et al.* 2020b). We did not excavate galleries or rear beetles from the trees and therefore were not able to determine beetle species at different heights on the trees or how many galleries were successfully established with brood. Additional research could help to determine whether beetle communities exhibit vertical stratification within infected trees, different species produce varying amounts of potentially infective frass, and native or non-native beetle species play distinctive roles in frass production.

We detected seven species of ambrosia beetles, plus Cerambycid spp. and *Hypothenemus* spp. in CVPT (Table 3). The most common species including *X. saxesenii*, *X. ferrugineus*, and *X. affinis* have previously been associated with ʻōhiʻa during ROD outbreaks (Curtis Ewing, University of Hawaiʻi at Mānoa, oral communication, 2018; Roy *et al*. 2020b). The relative importance of the different beetle species is unknown. The community composition of ambrosia beetle species at various ROD outbreaks across the Hawaiian archipelago is not fully understood. The small size of gallery entrances (≤2 mm; Figure 5), the thickness of bark, and the growth of moss, lichens, and other epiphytes on tree trunks in the tropical climate of

Hawai'i make a visual determination of the presence of beetle colonization and frass production difficult even for highly trained observers. Because the presence or absence of beetles is a key branch point in the current ROD management flow chart (Figure 1), beetle trapping in addition to searches for beetle galleries and frass may help to reduce the possibility that beetle colonization will be overlooked.

Efficacy of Bifenthrin in Preventing Ambrosia Beetle Attacks

Given the large number of trees that were cut, tarping was not a realistic option for reducing beetle colonization of felled trees. Therefore, a single treatment with bifenthrin was applied by land managers near the end of the study in an attempt to reduce beetle attacks. We took advantage of this opportunity to monitor a small subset of control and treated bolts to determine the efficacy of bifenthrin at reducing ambrosia beetle gallery attacks.

Relative to control bolts, bifenthrin was effective at reducing beetle colonization for the first three weeks of the study. Similarly, bifenthrin was effective for two weeks against *Xyleborus glabratus* when applied under hot, humid summer conditions in Florida (Carillo *et al.* 2014). Redding *et al*. (2013) found inconsistent effectiveness of bifenthrin against ambrosia beetle attacks in different midwestern states, but the product was effective for completely suppressing the establishment of *Xylosandrus germanus* in Ohio. While we found that bifenthrin was effective at reducing the number of beetle attacks relative to controls in our pilot study, the effect of the insecticide appeared to be short-lived in addition to the reduced attractiveness of the cut ʻōhiʻa to the beetles over time. Because bifenthrin is a potent toxin to aquatic ecosystems (Hoagland and Drenner 1993) and likely has many non-target effects on native invertebrates, the use of this product in Hawaii may be restricted in many forested habitats. Therefore, it may be prudent to investigate other avenues for beetle control, including the use of attractants and repellants.

CONCLUSIONS

No differences in cumulative airborne detections of *Ceratocystis* DNA were found between treatment and control areas and between management and post-management monitoring periods. However, we found some evidence that cumulative detections of *Ceratocystis* DNA in PES reached a plateau after most infected trees were felled. Detections once again increased, possibly due to standing, infected, yet asymptomatic trees, and also symptomatic trees that were not felled due to hazards. In support of this explanation, post-treatment monitoring of the site has documented continued tree mortality after the management operation was completed (Ryan Perroy, University of Hawai'i at Hilo, oral communication, 2020). Our results highlight the importance of identifying incipient outbreaks that involve only a few trees. Once outbreaks become too large, felling operations may only slow, rather than prevent the spread of disease. Because it may take anywhere from two months to over a year for a recently infected tree to exhibit outward symptoms, early detection tools such as detector dogs, portable hand-held devices for measuring volatile compounds released by infected trees, or hyperspectral monitoring from airborne platforms followed by management efforts could greatly decrease the spread of ROD. These data also highlight the challenges of landscape-scale forest management and the continued urgency for innovative management tools and methods.

Cross-vane panel traps may provide an alternative to inspecting trunks of symptomatic trees for beetle galleries and frass, particularly when beetle presence is difficult to detect (e.g., the bark is thick and shaggy or covered with moss and lichens). Accurate and early detection of beetles

is of high importance in the ROD management flowchart (Figure 1), and if the presence of beetles is missed, it may delay critical management decisions and actions. Hence deployment of CVPT in areas with ROD mortality may help to confirm the presence of target beetle species, particularly because the distribution of ambrosia beetle species throughout the Hawaiian landscape is largely unknown.

Both wind and rainfall may be important in mediating the spread of infective beetle frass and wood particles associated with tree cutting. Dry, windy conditions will likely promote the spread of airborne particulates while rainfall may reduce the spread by removing particulates from the air column. To be most effective, management operations could be timed for rainy days with minimum wind. While there is still no definitive evidence that viable, airborne beetle frass containing *Ceratocystis* propagules travels in the wind column (only *Ceratocystis* DNA), the use of PES may be a useful monitoring tool for detecting the presence of the fungi in forest tracts that are difficult to access. Environmental monitoring of streams may also be a useful tool for detecting the presence of the disease in remote locations, as the diseases may behave similarly to *Ceratocystis platani* (Ocasio-Morales *et al.* 2007).

Ambrosia beetles colonize ʻōhiʻa from the ground to the highest branches in the canopy but are most common in larger trunks near the ground. Given that *Ceratocystis* has been documented in ʻōhiʻa from ground level to canopy (Lisa Keith, USDA-ARS-DKI-PBARC, oral communication, 2020), the production of frass in the canopy may enhance the spread of potentially infective propagules in the wind column. When infected trees are felled, the use of pesticides such as Bifen I/T may be an effective yet short-lived alternative to tarping to reduce initial beetle attack and colonization, although non-target effects of the pesticide on aquatic organisms and native insects may limit its usefulness. Further research using varying insecticide formulations and under multiple environmental conditions is necessary before definitive conclusions can be made.

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LITERATURE CITED

Abbott, I. A. 1992. Lāʻau Hawaiʻi: Traditional Hawaiian uses of plants. Bishop Museum Press, Honolulu, Hawaii, USA. 163 pp.

Atkinson, C. T., K. Roy, and C. Granthon. 2019. Economical sampler designs for detecting airborne spread of fungi responsible for rapid ʻōhiʻa death. Hawaiʻi Cooperative Studies Unit Technical Report HCSU-087. University of Hawaiʻi at Hilo, Hawaii, USA. http://hdl.handle.net/10790/4568

Barnes, I., A. Fourie, M. J. Wingfield, T. C. Harrington, D. L. McNew, L. S. Sugiyama, B. C. Luiz, W. P. Heller, and L. M. Keith. 2018. New *Ceratocystis* species associated with rapid death of *Metrosideros polymorpha* in Hawaiʻi. Persoonia 40:154–181.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48. http://dx.doi.org/10.18637/jss.v067.i01.

Carrillo, D., J. H. Crane, and J. E. Peña. 2014. Potential of contact insecticides to control *Xyleborus glabratus* (Coleoptera: Curculionidae), a vector of laurel wilt disease in avocados. Journal of Economic Entomology 106:2286–2295.

Emerson, N. B. 2005. Pele and Hiʻiaka: A myth from Hawaiʻi. Revised edition. Edith Kanakaʻole Foundation, Hilo, Hawaii, USA.

Fortini, L. B., L. R. Kaiser, L. M. Keith, J. Price, R. F. Hughes, J. D. Jacobi, and J. B. Friday. 2019. The evolving threat of rapid ʻōhiʻa death (ROD) to Hawaii's native ecosystems and rare plant species. Forest Ecology and Management 448:376–385.

Fox, J., and S. Weisberg. 2018. Visualizing fit and lack of fit in complex regression models with predictor effect plots and partial residuals. Journal of Statistical Software 87:1–27. DOI: 10.18637/jss.v087.i09.

Gon, S. M., A. Allison, R. J. Cannarella, J. D. Jacobi, K. Y. Kaneshiro, M. H. Kido, M. Lane-Kamahele, and S. E. Miller. 2006. A GAP analysis of Hawaii: Final report. U.S. Department of the Interior, U.S. Geological Survey, Washington, D.C., USA.

Hawaiʻi Permanent Plot Network. 2018. Climatological data summaries. Retrieved from Ostertag, R., S. Cordell, T. Giambelluca, C. Giardina, C. Litton, M. Nullet, and L. Sack on May 15, 2018.

Heller, W. P., and L. M. Keith. 2018. Real-time PCR assays to detect and distinguish the rapid ʻōhiʻa death pathogens *Ceratocystis lukuohia* and *Ceratocystis huliohia*. Phytopathology 108:1395–1401. DOI: 10.1094/PHYTO-09-17-0311-R.

Hoagland, K. D., and R. W. Drenner. 1993. Freshwater community responses to mixtures of agricultural pesticides: effects of atrazine and bifenthrin. Environmental Toxicology and Chemistry 12:627–637.

Hughes, M. A., J. Juzwik, T. C. Harrington, and L. M. Keith. 2020. Pathogenicity, symptom development, and colonization of *Metrosideros polymorpha* by *Ceratocystis lukuohia*. Plant Disease 104:2233–2241. DOI: 10.1094/PDIS-09-19-1905-RE.

Keith, L. M., R. F. Hughes, L. S. Sugiyama, W. P. Heller, B. C. Bushe, and J. B. Friday. 2015. First report of *Ceratocystis* wilt on ʻōhiʻa (*Metrosideros polymorpha*). Plant Disease 99: 1276. DOI: 10.1094/PDIS-12-14-1293-PDN.

Kimmet, T. 2016. Hawaii Island, Hawaiian Islands, Orthoimagery Mosaic, Version 3. USDA-NRCS-National Geospatial Center of Excellence. Available at: https://geoportal.hawaii.gov/datasets/740cfa2888974c50a722e422831d312b

Luchi, N., L. Ghelardini, L. Belbahri, M. Quartier, and A. Santini. 2013. Rapid detection of *Ceratocystis platani* inoculum by quantitative real-time PCR assay. Applied Environmental Microbiology 79:5394–5404.

Marsaglia, G., W. Wan Tsang, and J. Wang. 2003. Evaluating Kolmogorov's distribution. Journal of Statistical Software 8:18. DOI: 10.18637/jss.v008.i18.

Mazerolle, M. J. 2019. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 2.2-2. https://cran.r-project.org/web/packages/AICcmodavg/index.html

Mueller-Dombois, D., J. D. Jacobi, H. J. Boehmer, and J. P. Price. 2013. ʻŌhiʻa lehua rainforest: Born among Hawaiian Volcanoes in isolation: The story of a dynamic ecosystem with relevance to forests worldwide. Friends of the Joseph Rock Herbarium, Middletown, Delaware, USA. 292 pp.

Ocasio-Morales, R. G., T. Tsopelas, and T. C. Harrington. 2007. Special report: Origin of *Ceratocystis platani* on native *Platanus orientalis* in Greece. Plant Disease 91:901–904.

Pukui, M. K. 1983. ʻŌlelo noʻeau. Bernice P. Bishop Museum Press, Honolulu, Hawaii, USA.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Redding, M. E., J. B. Oliver, P. B. Schultz, C. M. Ranger, and N. N. Youssef. 2013. Ethanol injection of ornamental trees facilitates testing insecticide efficacy against ambrosia beetles (Coleoptera: Curculionidae: Scolytinae). Journal of Economic Entomology 106:289–298.

Roy, K., C. Granthon, R. W. Peck, and C. T. Atkinson. 2020a. Waipunalei ROD Management 2017–2018. U.S. Geological Survey data release. https://doi.org/10.5066/P9THTCOX

Roy, K., C. P. Ewing, M. A. Hughes, L. Keith, and G. M. Bennett. 2019. Presence and viability of *Ceratocystis lukuohia* in ambrosia beetle frass from rapid ʻōhiʻa death-affected *Metrosideros polymorpha* trees on Hawaiʻi Island. Forest Pathology 49:e12476. DOI: 10.1111/efp.12476.

Roy, K., K. A. Jaenecke, and R. W. Peck. 2020b. Ambrosia beetle (Coleoptera: Curculionidae) communities and frass production in ʻōhiʻa (Myrtales: Myrtaceae) infected with

Ceratocystis (Microascales: Ceratocystidaceae) fungi responsible for Rapid ʻŌhiʻa Death. Environmental Entomology. https://doi.org/10.1093/ee/nvaa108

Samuelson, G. A. 1981. A synopsis of Hawaiian Xyleborini (Coleoptera: Scolytidae). Pacific Insects 23:50–92.

Smathers, G. A., and D. Mueller-Dombois. 1974. Invasion and recovery of vegetation after a volcanic eruption in Hawaii. United States National Park Service Scientific Monograph Series 5. 129 pp.

Takahashi, T., M. Tamura, Y. Asami, E. Kitamura, K. Saito, T. Suzuki, S. N. Takahashi, K. Matsumoto, S. Sawada, E. Yokoyama, and T. Takasu. 2008. Novel wide-range quantitative nested real-time PCR assay for *Mycobacterium tuberculosis* DNA: Development and methodology. Journal of Clinical Microbiology 46:1708–1715.