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Vacuum Steam Treatment of *Metrosideros polymorpha* Logs for Eradication of *Ceratocystis huliohia* and *C. lukuohia*

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Abstract

A new and devastating disease, rapid ohia death (ROD), in Hawaii led to a state quarantine that regulates interisland transport of ohia wood and plant material to prevent spread of the causal pathogens. Heat treatments of ohia logs in commercial trade were considered for phytosanitary treatment. Vacuum steam (VS) was evaluated for its ability to eradicate the pathogens, *Ceratocystis lukuohia* and *C. huliohia*, in main stem logs from ROD-affected forest trees. Replicate loads of three debarked logs (24 to 43 cm in diameter, 1.7 to 2.0 m long) were VS treated at 56°C for 30 min (five loads) or 60°C for 60 min (four loads) at a sapwood depth equal to 70% of log radius. Percentage isolation of *Ceratocystis* from VS and ambient temperature logs before treatment and summarized by source tree ranged from 12 to 66% and 6 to 31% based on carrot baiting assays of tissue taken from outer and inner sapwood, respectively. No viable *Ceratocystis* was detected in sapwood locations for the 60°C/60 min schedule or inner locations for the 56°C/30 min schedule after treatment. Only one subsample (0.48%, *n* = 208) of the latter schedule treatment yielded *Ceratocystis*. Time needed for treatment ranged from 7.4 to 15 h for the 56°C/30 min schedule and from 8.6 to 19.2 h for the 60°C/60 min schedule. These results demonstrate that VS is an effective and efficient method for treating large-diameter ohia logs that mill owners and regulatory plant pathologists may consider for use in Hawaii.

Keywords: phytosanitary treatment, rapid ohia death, regulatory plant pathology

An emerging disease, rapid ohia death (ROD), threatens the health and survival of the keystone forest tree species *Metrosideros polymorpha* (ohia) in the Hawaiian archipelago (Mortenson et al. 2016). After the first visual detections between 2009 and 2012 of what is now known as ROD, the putative causal agent was determined to be *Ceratocystis fimbriata* in 2014 (Keith et al. 2015). However, further morphological observations and fungal interfertility and phylogenetic analyses have led to the differentiation of two distinct pathogens: *Ceratocystis lukuohia* I. Barnes, T.C. Harr., & L.M. Keith and *C. huliohia* I. Barnes, T.C. Harr., & L.M. Keith (Barnes et al. 2018). The first causes a systemic vascular disease and is associated with a rapid wilt of the entire crown of an infected ohia (Hughes et al. 2020), and the second causes distinct but often extensive, cankers (Juzwik et al. 2019a). Multiple cankers caused by the latter are also capable of causing tree death, albeit over a longer timeframe than the wilt pathogen. Currently, *C. lukuohia* is known to occur on Hawaii Island and Kauai, whereas *C. huliohia* has been confirmed on Maui and Oahu in addition to Hawaii Island and Kauai (College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa 2021). In an early attempt to prevent spread of the

pathogens from Hawaii Island to the rest of the state, the Hawaii Department of Agriculture enacted quarantine regulations restricting interisland movement of any ohia plant parts and soil associated with the plant species (Hawaii Department of Agriculture Amendment to chapter 4-72, amendment §4-72-13). Off-island shipment of ohia logs has been permitted on a log-by-log basis since 2016 if a log is found to be free of ROD pathogen DNA based on quantitative PCR (qPCR) assay (Heller and Keith 2018) of multiple samples taken along the length of the log. Besides its inability to distinguish between viable and nonviable pathogen propagules, this testing process is time consuming and delays commercial log sales for the forest industry in Hawaii.

Ohia logs (locally referred to as poles and posts) of diameters from 8 to 50 cm and of various lengths (usually ranging from 3 to 6 m) are purchased for such uses as decorative poles and beams, fences, rustic bridges, and building structural supports. Price per log is based on base diameter and log length, with costs increasing with size. Although prices vary, the larger logs command prices of thousands of dollars each. Logs are debarked early in the production process and often sold "green" (i.e., with a high moisture content).

Kiln-heating treatment at 56°C of prohibited wood materials was suggested as an eradicative treatment in the original quarantine; however, no certified heat treatment schedules were available at the time. In response to the need, research efforts were initiated in 2017 to evaluate three different heat treatments and one chemical treatment potentially suitable for debarked ohia logs. Because both ROD-associated *Ceratocystis* spp. colonize the xylem (Hughes et al. 2020; Juzwik et al. 2019a), depth of effective treatment was an important consideration. For mill owners in the state, cost of treatment and availability of equipment needed for effective treatment are of concern. Chemical dip diffusion treatments were appealing because they are simple to apply, require minimal capital investment for mill owners, and are low in cost. However, incomplete eradication of *C. lukuohia* in the inner sapwood was found in log sections (9 to 17 cm in diameter, 1.3 m long) immersed in a borate/quaternary ammonia solution with a 10-week diffusion period (Hughes et al. 2021).

Vacuum steam (VS) was recently evaluated for use with large-diameter (41 to 61 cm), 1.9-m-long logs cut from *Quercus rubra* trees that had recently wilted because of infection by *Bretziella fagacearum*

(syn. *Ceratocystis fagacearum*), the oak wilt fungus. Two treatment schedules (60°C for 60 min and 56°C for 30 min) were both found to eradicate living propagules of *B. fagacearum* from the sapwood; furthermore, DNA of the fungus was degraded by the treatments (Juzwik et al. 2019b). Loads of three logs were tested in each trial. Treatment for 6 to 10 h was needed to achieve the temperature and time specifications of the administered regimen. A previously published study (Chen et al. 2017) found that the VS process had minimal deleterious effect on product (i.e., veneer) quality of five hardwood species tested. Because of similarities of *C. lukuohia*-caused wilt of ohia to *B. fagacearum*-caused wilt of oaks, VS holds promise for effective eradicative treatment of *Ceratocystis* species of ohia.

Outdoor experiments including a portable VS system were undertaken to evaluate the ability of the VS process to kill ROD pathogens in colonized, debarked logs obtained from naturally infected *M. polymorpha* of large diameter (24 to 43 cm). The specific objectives were to evaluate the ability of VS heat treatments to eradicate viable propagules of *C. lukuohia* or *C. huliohia* at two sapwood depths, document the temperature profile of the treated logs over the time course of the experiments, document the energy and time needed to treat each load, monitor for any changes in log quality (before vs. after treatment), and evaluate potential for pathogen recolonization of treated wood.

Materials and Methods

Study trees. Naturally infected, ROD-symptomatic ohia trees located on woodlands used for cattle grazing in Waipunalei, North Hilo District at elevation 530 m and on a private ranch in the District of Kau (location withheld to protect identity of the landowner) on Hawaii Island were selected as sources of study logs between March and September 2018. During the initial scouting for trees at Waipunalea in March 2018, the identified candidate trees were in the early stages of crown wilt (≥25% severity) (data not shown) and had progressed to a range of 30 to 100% by the time of their last rating date (Supplementary Table S1). Scouting for study trees at the Kau site was conducted in July and November 2018. The time from their last crown rating (80 to 100% severity) to time of log treatment ranged from 73 to 215 days. Presence of *C. lukuohia* or *C. huliohia* in the main stems was verified from drill-shaving samples from the xylem and subjected to qPCR assay according to published protocols (Heller and Keith 2018). The Waipunalei site is on the island's north lower flank of Mauna Kea and receives 4,311 mm of rain annually (Giambelluca et al. 2013). In contrast, the Kau ranch site is on the island's south side and receives 1,616 mm of annual rainfall. Diameters of the selected ohia trees ranged from 33 to 64 cm and 32 to 58 cm at 1.4 m stem height at the Waipunalei and Kau ranch sites, respectively. Permission to harvest trees on each site was obtained from current landowner or land lease holder.

Fifteen trees on the Waipunalei site that were determined to be positive for ROD *Ceratocystis* DNA via qPCR were felled in late December 2018. Twenty-nine candidate logs (2.1 m long) were cut from the main stems on site, and the logs were transported to a University of Hawaii Manoa property in Hilo for temporary outdoor storage. Five trees that tested positive for *Ceratocystis* spp. DNA on the Kau ranch site were felled in mid-January 2019, similar-sized logs were removed from main stems, and 12 candidate logs were transported to the same site in Hilo. Overall, 41 logs were obtained as candidates for treatment, although not all were used in the trials.

Log preparation, measurements, and experimental design. In late January 2019, the logs were moved to a treatment site at the U.S. Department of Agriculture Agricultural Research Service Pacific Basin Agricultural Research Center facility in Hilo. There they were sorted into three treatments (two levels of VS and the ambient temperature control), with attempts made to select three logs of similar diameters that had originated from different trees for VS treatment. In addition, logs with obvious injury from the harvesting process or with preexisting defects were excluded from use. Because commercial ohia logs are debarked before processing, the final selected logs were debarked with high pressure water and drawknives. A 28-cm-long section was cut from the ends of each log, cut

surfaces were painted with liquid paraffin (Thompson's Water Seal, The Thompson's Company, Cleveland, OH), and discs were labeled and stored at 5°C until further processed for pretreatment assays for viable *Ceratocystis*. In addition, for each log, one cross-sectional disc (7.6 cm thick) was cut from the larger-diameter end of the log, placed in a polybag, and used for pretreatment sapwood moisture content determination. The moisture content discs were processed as follows within 1 h of collection: A pie-shaped section was cut from each disc, weighed (top-loading balance), placed in a labeled paper bag, and left in a drying oven (103°C) until constant weight was obtained.

End diameters and final length of each prepared log were then measured and recorded. Liquid paraffin was painted onto the ends of each log and allowed to dry. Weight of each log was obtained immediately before treatment. Two heat treatment schedules (60°C/60 min and 56°C/30 min) were evaluated, with four or five replicate chamber loads (tests) used per schedule. The unequal numbers of replicates were caused by the unexpected failure of the diesel generator during the last 60°C/60 min test. Four logs were left untreated to determine whether ambient outdoor conditions reduced fungal viability over the course of a trial. For a control trial (two logs per ambient temperature trial), pretreatment samples were taken as previously described, logs were stored under shade, and posttreatment samples removed 24 h later.

Obtaining sample discs for fungal assay. Discs (7 to 8 cm thick) for pretreatment fungal assays were obtained with a bandsaw. The end sections obtained from each log were placed in a jig, and cross-sectional cuts were made with a bimetal blade on a large bandsaw (Model JWBS-18-3 18" Bandsaw, 3 HP, JET Tools, Inc., La Vergne, TN). All sawdust was removed from the equipment and floor between processing of each log section. In addition, the blade, jig, and cutting surface were sterilized with 70% ethanol in between sections to minimize fungal contamination between sampled discs. The discs were labeled, placed in polybags, and stored at 5°C until further processed.

Similar methods were used to obtain sample discs from posttreatment VS and control logs. End sections (28 cm long) were obtained from treated logs after they had cooled to a safe handling temperature after their removal from the VS chamber. Sample discs for fungal assay were obtained and stored until further processed as previously described. In addition, one disc (7.6 cm thick) was obtained from each posttreatment log for moisture content determination via the previously described methods.

Fungal assays. To further minimize the possibility of contamination between pre-VS treatment (or control discs) and the post-VS-treatment discs, all posttreatment disc subsamples were prepared and assayed first. Using a circular template, we drew lines delineating 16 pie-shaped sections on each disc. Using the bandsaw, we made cuts along each line from the perimeter to approximately 5 or to 8 cm inward toward the geometric center of a disc. The disc was then stored in a polybag at 5°C and taken to the pathology laboratory for assay. Carrot baiting (Moller and DeVay 1968) was used to estimate frequencies of viable *Ceratocystis* presence in alternating outer (1 to 3 cm deep) and inner (4 to 6 cm deep) sapwood locations of each disc. A 2-cm-wide sterile chisel was used to cut thin wafers of wood at each depth and location. Two or three wafers were misted with sterile water if they appeared dry, placed between two carrot discs (0.5 to 1.0 cm thick), bound together with a strip of laboratory film, placed in a polybag, and incubated at ambient temperature (about 24°C) and lighting conditions for up to 30 days. Baits were considered positive for either *Ceratocystis* species if sexual fruiting bodies (perithecia) were visible on the wood tissue or surface of a carrot disc. If only grayish mycelia or immature perithecia were observed, a portion of the colonized carrot piece was placed in a sterile microcentrifuge tube for DNA extraction and subjected to qPCR assay (Heller and Keith 2018). In addition, a small subsample of carrot baits (based on stratified random sampling) also was subjected to qPCR to identify *Ceratocystis* species present.

Log temperature and energy use monitoring. After individuals of a three-log load were weighed and placed on a wood pallet for insertion into the vacuum chamber, Omega K type thermocouple

wires (TT-K-24; 260°C max) were inserted into predrilled holes located at 1/4, 1/2, and 3/4 along the length of each log (Supplementary Fig. S1). The holes were drilled to a depth of 70% of the log radius at each point. In addition, one hole was drilled to the geometric center of the log at its midpoint, and one thermocouple was affixed to the log surface at the midpoint. A final thermocouple was inserted into one end of the log to a depth equal to 70% of the log radius for the end probed. After a thermocouple wire was inserted to the bottom of a hole, and the hole was plugged with pliable epoxy resin (plumber's putty) to block steam from entering it. The temperature probe wires were then connected to the data acquisition system to allow continuous data recording. Voltage and current of each phase were measured every minute during each trial with an energy meter (ELITEpro XC, Dent Instrument, Bend, OR) and data retrieved with Elog software (Dent Instrument). The kilowatt hours were calculated by multiplying voltage by current. The measured wattages were recorded and converted to kWh per kg of wood treated.

VS treatment. The portable VS unit includes an electric steam generator with a 100-kW boiler (Reimers Electra Steam Inc., Model RX100C3F, Clear Brook, VA), a 5-hp dry screw vacuum pump (Busch LLC, Virginia Beach, VA), and a custom-built vacuum chamber (1.5 × 1.5 × 3.0 m capacity) (Vacutherm Inc., Warren, VT). The unit was secured in position in the back portion of a 6.5-m-long enclosed trailer (see White et al. 2017). The thermocouple wires exited a side wall of the chamber in a narrow port that was sealed, and the wires connected to a computer equipped with data acquisition software (LabVIEW, National Instruments Corp., Austin, TX) that allowed real-time monitoring of temperatures. Once a test load was inserted into the chamber and the door sealed, the vacuum treatment was initiated. After a vacuum of 100 mm Hg was reached, saturated steam (85°C) was introduced to the chamber until the chamber reached 85°C. This chamber temperature was maintained throughout the treatment cycle. Vacuum level varied between 400 and 600 mm Hg during treatment, depending on the duration of the treatment cycles. Temperatures of the targeted log locations and of the ambient chamber were monitored throughout each trial until the target temperature was reached for all the probes inserted at 70% of each log's radius depth. The trials were then stopped when the prescribed temperature (either 56 or 60°C) was achieved at the target depth. The steam was then shut off, and the target temperature held for the prescribed time (30 min for 56°C or 60 min for 60°C). At the end of the specified time, the remaining vacuum was released, and the chamber doors opened to allow evacuation of the steam and condensate.

Log quality assessment. Visual observations for each log were recorded in writing and with photographs to document the effects of treatment on log color and structural degradation, such as end-checking and splitting.

Fungal inoculation and colonization of posttreatment logs. To assess whether VS-treated wood was vulnerable to recolonization by *C. lukuohia* and *C. huliohia*, we exposed logs to the fungi in a post-VS treatment artificial assay. Fungal isolates of *C. lukuohia* (14-1-1) and *C. huliohia* (16-8) were grown on 10% V8 media for 7 days, plates flooded with sterile water, and propagules were collected into beakers. Sterile filter paper discs (Whatman no. 1, 42.5 mm diameter) were soaked in the fungal suspension for 1 min, laid onto agar media, and incubated for 6 days at 25°C until fully colonized (Keith et al. 2015).

Four VS-treated logs from each treatment schedule (30 min for 56°C; 60 min for 60°C) were randomly selected 2 weeks after VS treatment. Each filter paper inoculation site (8 × 8 cm square) was prepared by having the outermost layer of wood (1 to 2 mm) removed by a sterile drawknife blade and cut surface sprayed with sterile water to rehydrate the site. Per each log, a single *C. lukuohia*, *C. huliohia*, and water-soaked (negative control) filter paper disc was stapled onto the freshly prepared wood surface, resprayed with water, and allowed to incubate outdoors with sun and rain exposure (mean daily highs of 26.4°C) for 14 days. Inoculation sites were separated by 20 cm along the log length and were watered every 24 h with a watering can unless rain occurred. To validate inoculum viability,

two paper discs per treatment (*C. lukuohia*, *C. huliohia*, and negative control) were placed between two fresh carrot slices and held in a plastic sandwich bag next to the logs. After 2 weeks, filter paper discs were removed from the logs, and the wood surface underneath visually inspected for *Ceratocystis* fungal growth. An 8 × 8 cm square and approximately 1-cm-thick section were then removed from each inoculation site. Thin wood strips were removed with a flame-sterilized chisel and carrot baited as above.

Data summarization and analyses. *Ceratocystis* spp. isolation data were summarized by sapwood depth and tree harvest site. A generalized linear mixed effects model (Agresti 2002) was used to investigate effects of these variables on isolation frequencies, particularly site and wood depth. The generalized linear mixed effect model has the form:

$$Y_{ijk} \sim Bernoul(P_{ijkl})$$
$$\text{Logit}\,[P(Y_{ijk}) = 1] = \mu + S_i + D_j + SD_{i\,j\alpha k} + \gamma_{1\,(k)}$$
$$\alpha_k \sim N\,(0,\,\sigma_{tree})\ and\ \gamma_{1(k)} \sim N\,(0,\,\sigma^2_{log}),$$

where P is the probability of detecting the fungus, μ is the overall mean, S is the site where trees were obtained (Kau or Waipunalei), D is the sapwood depth (inner or outer sapwood), α is the error associated with tree, and γ is the error associated with log. All calculations were performed in R (version 1.0.143; R Foundation for Statistical Computing, Vienna, Austria). The model was run as a generalized mixed effects model with lme4 (Bates et al. 2015). Analysis of variance (type II Wald χ^2 tests) for the mixed effects model of pretreatment isolation data for all logs was conducted to investigate variable effects on the likelihood of fungus detection. Odds ratios and estimated probabilities of detecting *Ceratocystis* spp. from the inner and outer sapwood of diseased trees were calculated with the lsmeans package (Lenth 2016). Temperature profiles for the monitored logs in each treatment trial were summarized graphically. Means and standard errors of wood moisture contents for all study logs were calculated for the pretreatment and posttreatment assessment dates.

Results

Characteristics of test logs. The dimensions of the test logs after their ends were sampled for fungal assay and wood content, but before they were subjected to VS or control treatments, ranged from 1.7 to 2.1 m in length (Supplementary Table S2). The diameters of the small end of each log ranged from 22 to 44 cm. Weights of the three logs in each load were determined immediately before the load was placed in the vacuum chamber. Logs of similar diameters were included in a load. The pre–VS treatment log weights ranged from 90 to 329 kg.

Pathogen presence in logs. *Ceratocystis* spp. were detected on carrot baits in sapwood for 27 of the 31 logs assayed before treatment. Subsamples from the four *Ceratocystis*-negative logs also did not yield the fungus in posttreatment assays. These particular logs were from four different trees in Waipunalei. When the *Ceratocystis*-positive, pretreatment logs ($n = 27$) were grouped by source tree, the mean rates of pathogen detection ranged from 12.5 to 65.6% of the outer sapwood assayed locations and from 6.2 to 31.2% of inner sapwood locations (Table 1). The highest grouped mean was for tree EB02, whose two logs also had the highest outer and inner sapwood detection levels of all study logs (Table 1). When all pretreatment logs ($n = 31$) were summarized by frequency of detection level according to sapwood depth, more logs had higher percentages of *Ceratocystis*-positive outer sapwood assay locations ($n = 16$) compared with inner ones ($n = 16$) (Fig. 1).

There was no difference in detection of *Ceratocystis* spp. from logs obtained from the two sites ($P = 0.5997$), based on results of the generalized linear effects model (Table 2). However, differences were found for probability of detection by depth of sapwood assayed. The estimated probability of viable pathogen detection for all pretreatment logs (combined sites) was higher for subsamples obtained from outer sapwood than those from inner sapwood (Table 3). Subsamples taken

from the inner sapwood were half as likely to yield *Ceratocystis* spp. compared with samples from the outer sapwood, based on results of odds ratio contrast analysis (odds ratio = 0.49; *P* = 0.0001).

When pretreatment detection data were summarized by treatment (VS treatment schedules; ambient temperature control), 25.6 to 26.6% of outer sapwood and 13.9 to 18.8% of inner sapwood locations on discs from all logs yielded *Ceratocystis* on carrot baits (Table 4). In comparison, no viable *Ceratocystis* was detected in outer and inner sapwood of logs after the 60°C/60 min VS treatment and inner sapwood of the 56°C/30 min treatment. Furthermore, only a single subsample (0.48%) (*n* = 208) from the outer sapwood yielded *Ceratocystis* on carrot baits after 56°C/30 min treatment. In contrast, viable pathogen levels were similar before and after 24 h of ambient temperature for control logs held outdoors under shade

Table 1. Frequencies (numbers and percentages) of assayed subsamples yielding *Ceratocystis lukuohia* or *C. huliohia* on carrot baits for the outer and inner sapwood of logs taken from naturally infected *Metrosideros polymorpha* trees at Waipunalei and Kau ranch sites, Hawaii Island, before vacuum steam or ambient temperature (control) treatment

| | | | *Ceratocystis*-positive carrot baits by sapwood depth | | | |
| | | | Outer | | Inner | |
Tree ID[a]	Number of logs[b]	Number of assayed locations	Number	Percentage	Number	Percentage
W-T2	3	48	14	29.2	8	16.7
W-EB02	2	32	21	65.6	9	28.1
W-202	2	32	6	18.8	4	12.5
W-203	2	32	7	21.9	6	18.8
W-204	1	16	3	18.8	2	12.5
W-205	2	32	7	21.9	3	9.4
W-206	2	32	8	25.0	4	12.5
W-209	1	16	4	25.0	3	18.8
W-210	1	16	2	12.5	1	6.2
K-269	2	32	4	12.5	3	9.4
K-270	1	16	7	43.8	5	31.2
K-271	3	48	11	22.9	4	8.3
K-272	5	80	18	22.5	12	15.0

[a] Letter preceding number indicates site where tree was located: W, Waipunalei; K, Kau.
[b] Sapwood subsamples from four pretreatment logs (one from each of Waipunalei trees 202, 203, 204, and 210) did not yield *Ceratocystis* in carrot-baiting assays. These *Ceratocystis*-negative logs were not used in calculating frequencies for this table.
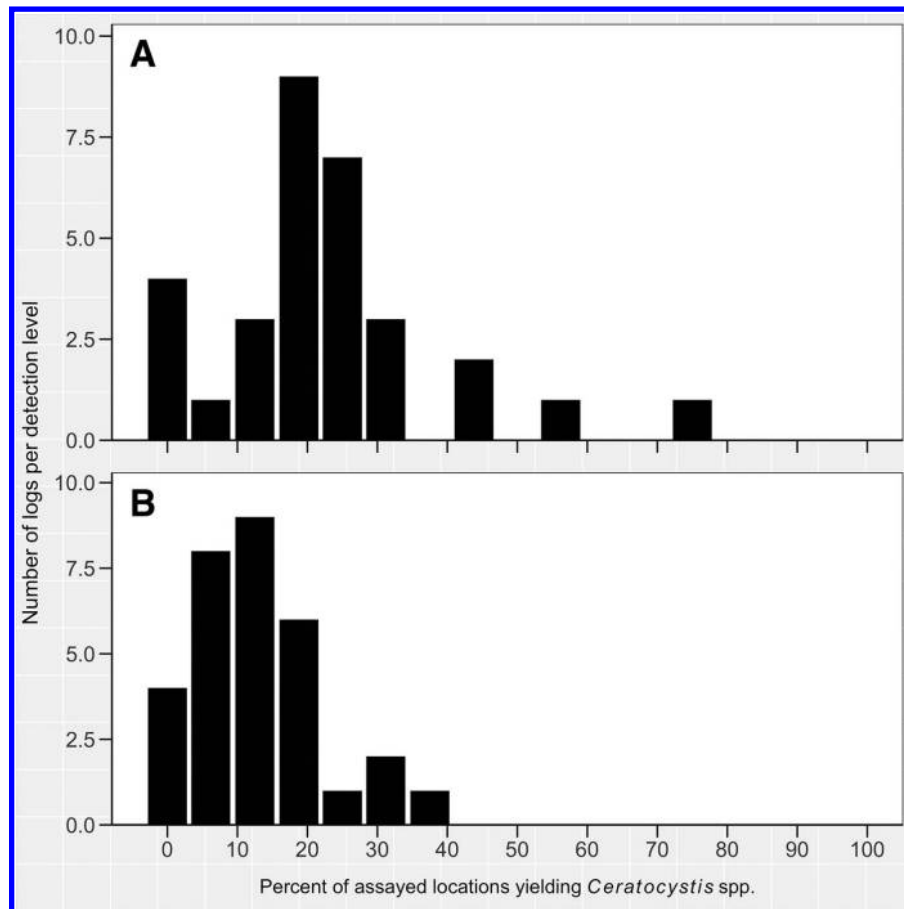
Fig. 1. Numbers of pretreatment logs (*n* = 31) yielding rapid ohia death *Ceratocystis* spp. by sapwood assay depth (**A**, outer sapwood; **B**, inner sapwood) of logs cut from naturally infected *Metrosideros polymorpha* trees. Detection level is displayed as the percentage of assayed disc sampling location (16 per log) yielding the pathogens. Numbers are based on isolation results of two discs for each log. Wood tissues were assayed via the carrot-baiting technique described by Moller and DeVay (1968). Data for the two log-source sites are combined because no difference (*P* = 0.5997) was found in fungus detection between them.

(Table 4). Only *C. lukuohia* was detected in the 28 carrot baits sub-sampled (from 432 total baits) and subjected to qPCR analysis.

Temperatures achieved, time, and energy needed for VS treatment. Three temperature probes were placed at a depth of 70% of calculated log radius in three locations along the length of the log. Temperatures in these locations were continuously monitored during treatment to determine when to begin the prescribed treatment time. The monitored placement depths ranged from 8.4 to 13.3 cm for logs in the five 56°C/30 min schedule trials and 8.9 to 14.4 cm in the four 60°C/60 min trials. The range of times for the probes to reach 56°C and hold for 30 min in five replicate trials was 442 to 924 min (7.4 to 15.4 h) (Table 5). In comparison, the range of times for the probes to reach 60°C and hold for 60 min in four replicate trials was 515 to 1,151 min (8.6 to 19.2 h). Representative temperature profiles based on temperatures recorded during a 56°C/30 min and a 60°C/60 min treatment are shown in Figure 2. Energy needed for the shorter treatment schedule ranged from 58 to 112 kWh or 0.15 to 0.26 kWh/kg on a weight basis (Table 5). The energy requirements for the longer schedule ranged from 73.8 to 121.4 kWh or 0.06 to 0.18 kWh/kg on a weight basis.

Sapwood moisture content. Log weights were recorded after VS treatment once logs were sufficiently cooled to handle safely or after 24 h for control logs to obtain an indirect measure of any changes in whole log moisture content. No or negligible changes were found for VS and control logs (Supplementary Table S2). Moisture content of sapwood in subsampled portions of pretreatment VS log discs ranged from 52.9 to 97.4% with a mean of 71.3%, as calculated on a dry weight basis (data not shown). Similar moisture content measurements of sapwood for discs taken from 27 logs after treatment (when cooled sufficient to handle) ranged from 51.0 to 94.2%, with a mean of 69.8%. On average, moisture content decreased by 1.5% after VS treatment.

Posttreatment log quality. Both ends of each study log were visually evaluated and photographed before and within 2 h after VS treatment to document physical conditions. Closer visual evaluations of logs were conducted 2 days after treatment ended for each load. Small end-splits were observed on ends of two pretreatment logs. Small to medium-length (half to three quarters of a log's radius measurement) end split lines were observed on most of the logs. However, the splits did not extend >15 cm deep into the logs. No change in log color was observed in posttreatment logs.

***Ceratocystis* inoculations of treated logs.** Carrot baits with *Ceratocystis*-infused filter paper discs applied had visible fungal mycelia and perithecia within 5 days, indicating that viable inoculum was used in the assay. No fungal growth was visible on the wood surface of any VS-treated logs, similar to the sterile water filter papers (negative control). Carrot baits from excised wood strips were all negative for *Ceratocystis* growth, regardless of fungal species.

Discussion

The VS process with a 56°C/30 min schedule was effective in eradicating *Ceratocystis* in >99.5% of sapwood samples assayed, whereas the 60°C/60 min schedule resulted in total eradication of

Table 2. Coefficients of the generalized linear effects model fit to the *Ceratocystis* spp. carrot-baiting assay data for inner and outer sapwood depths of discs sampled before vacuum steam or ambient temperature treatments[a]

Variable	Coefficient estimate	SE	P
Intercept	−1.8739	0.3946	<0.0001
Site: Waipunalei	−0.2529	0.4818	0.5997
Depth: outer sapwood	0.6461	0.2865	0.0241
Site × depth	0.1244	0.3629	0.7318

[a] Discs were from logs cut from main stems of 13 naturally infected *Metrosideros polymorpha* trees at two sites on Hawaii Island. Kau ranch site and inner sapwood are the reference levels.

Table 3. Estimated probabilities of *Ceratocystis* spp. detection via carrot-baiting assay of sapwood subsamples from pretreatment logs cut from diseased *Metrosideros polymorpha* trees[a]

Tree source location	Sapwood depth	Estimated probability[b]	Standard error	95% confidence interval
Kau	Outer	0.225	0.064	(0.066, 0.246)
Kau	Inner	0.389	0.132	(0.124, 0.374)
Waipunalei	Outer	0.198	0.039	(0.132, 0.287)
Waipunalei	Inner	0.103	0.025	(0.063, 0.164)
Combined[c]	Outer	0.216	0.025	(0.077, 0.179)
Combined[c]	Inner	0.119	0.039	(0.149, 0.301)

[a] Probabilities are based on logistic regression of the interaction of tree source location (Kau or Waipunalei) and sapwood depth (outer or inner sapwood).
[b] Probabilities and confidence intervals are back transformed from the logit scale.
[c] Because the tree source site had no significant effect on the likelihood of fungus detection, it is more appropriate to use the estimated probabilities from the pooled data for predictive purposes.

Table 4. Numbers of assayed locations with subsamples yielding *Ceratocystis* spp. on carrot baits from the outer and inner sapwood of logs from naturally infected *Metrosideros polymorpha* trees at Waipunalei and Kau ranch sites, Hawaii Island, before and after vacuum steam treatment or exposure to ambient conditions for 24 hours

Treatment schedule[a]	Number of tests	Number of logs[b]	Number of assayed sapwood locations	Pretreatment: fungus-positive baits by depth		Posttreatment: fungus-positive baits by depth	
				Outer (number)	Inner (number)	Outer (number)	Inner (number)
56/30	5	13	208	54	29	1	0
60/60	4	10	160	41	23	0	0
Ambient/24	2	4	64	17	12	18	8

[a] Treatments include 56/30 = 56°C held for 30 min at threshold temperature at 70% radius depth; 60/60 = 60°C held for 60 min at threshold temperature at 70% radius depth; and Ambient/24 = Ambient temperature over 24 h at 70% radius depth.
[b] Three logs were used per treatment load for each test. However, subsamples from four logs collected before and after one 56/30 and two 60/60 vacuum steam tests did not yield *Ceratocystis* spp. in carrot-baiting and are excluded from this table.

Ceratocystis in sapwood of ohia logs colonized by the ROD pathogens. Specifically, 22 to 44 cm diameter (small end) logs from naturally infected ROD trees that were heat-treated to targeted depths of approximately 70% of log radius were evaluated. The targeted depths are greater than depths previously reported to yield *C. lukuohia* and *C. huliohia* in fungus colonization studies of naturally infected ROD trees (Hughes et al. 2020; Juzwik et al. 2019a). However, the sapwood depths (between 4 and 6 cm) from which inner wood samples were obtained for carrot-baiting assays in the current study were at least one half of the maximum depth of the targeted VS treatment depth; thus, fungus detection was not attempted at the predetermined threshold temperature depth. In comparison, the maximum temperatures reached at 6 cm would be greater than either the 56 or 60°C reached at a depth of 70% of the log radius (e.g., 12 cm for a 17-cm diameter log). Further refinement of the optimal depth for achieving the targeted temperature could be of value, particularly for purposes of reducing energy consumption for treatment. The targeted depth (5 cm) of threshold temperature used for treating *B. fagacearum* colonized oak logs was based on the depth to the generally obvious sapwood–heartwood boundary (Juzwik et al. 2019b).

Table 5. Vacuum steam treatment cycle time, heating rate, and energy usage for the two treatment schedules with threshold temperature at 70% of log radius depth in sapwood of *Metrosideros polymorpha* logs

Treatment schedule[a]	Test number[b]	Average initial log temperature (°C)	Total cycle time (min)	Heating rate (min/°C)	Log load weight (kg)	Energy use kWh	Energy use kWh/kg
56/30	1	24	442	13.8	312.4	58.00	0.1857
56/30	2	23	924	28	–[c]	112.05	–
56/30	6	27	474	16.3	332.9	73.67	0.2213
56/30	8	21	608	17.4	438.6	66.11	0.1507
56/30	9	20	499	13.9	296.6	75.76	0.2554
60/60	3	22	515	13.6	305.4	54.74	0.1792
60/60	4	25	1,151	32.9	865.5	121.43	0.06364
60/60	5	24	853	23.7	564.3	83.73	0.1484
60/60	7	25	767	21.9	517.1	73.81	0.1427

[a] Treatments: 56/30 = 56°C held for 30 min; 60/60 = 60°C held for 60 min. Threshold temperatures for treatments were at 70% of log radial depth. Initial vacuum 100 mg Hg and saturated steam 85°C.
[b] The three logs per test were obtained from trees that were naturally colonized by *Ceratocystis* spp. that cause rapid ohia death.
[c] Dashes indicate that log weights for three logs in the load were not taken.
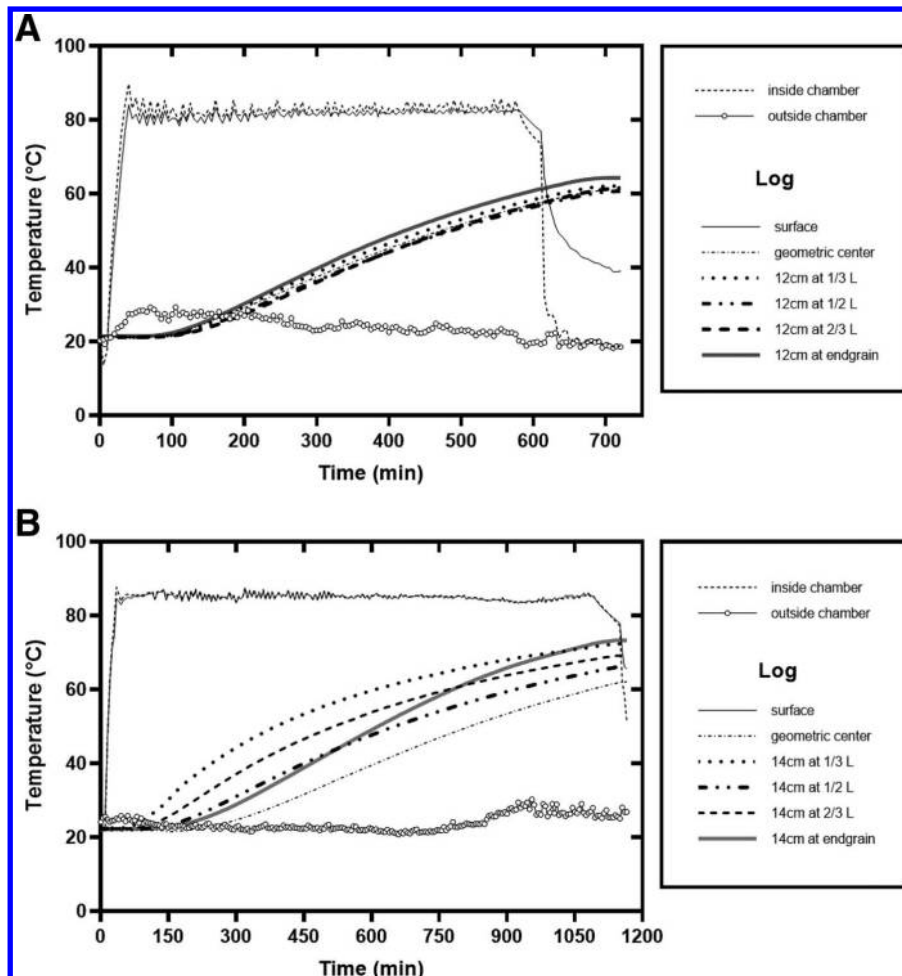
Fig. 2. Representative temperature profiles of *Metrosideros polymorpha* logs treated in the vacuum steam trials. **A,** 56°C for 30 min treatment of log 285 (Test 8); **B,** 60°C for 60 min treatment of log 49 (Test 4).

The sapwood–heartwood boundary in ohia is indistinct. Furthermore, dark red staining (reaction wood) in cut ends of main stem sections from ROD infected ohia trees often extends past 70% of the log radius and is not distinct from heartwood even though *Ceratocystis* may not be present.

The overall mean rates of *Ceratocystis* spp. detection were higher for the outer sapwood than the inner locations when logs were groups by source tree. The *Ceratocystis* associated with each symptomatic tree was determined when trees were being selected as sources for study logs. Eleven of the 13 trees from which the study logs came were infected with *C. lukuohia*. Thus, if one assumes most of the logs were colonized by *C. lukuohia*, the trend of higher pathogen isolation rates in the outer sapwood versus the inner is consistent with isolation depth of *C. lukuohia* from stained xylem found between 2 and 4 cm depth in earlier colonization studies of naturally infected *C. lukuohia* trees (Hughes et al. 2020). In the current study, pathogen detection levels were <32% of outer sapwood and <19% of inner sapwood for 85% of the *Ceratocystis*-positive logs. For isolation rates of *B. fagacearum* in pre-VS-treated *Q. rubra* logs from naturally infected trees, values ranged from 9 to 36% for outer and 5 to 16% for inner sapwood locations (Juzwik et al. 2019b).

Negligible loss of sapwood moisture (average 1.5%) was found for VS-treated ohia logs based on log disc samples. An even smaller loss (average <0.1%) in sapwood moisture content was found for VS-treated northern red oak logs colonized by *B. fagacearum* (Juzwik et al. 2019b) and an increase (2 to 4%) in sapwood moisture content for VS-treated *Juglans nigra* logs colonized by the bark canker pathogen *Geosmithia morbida* (Juzwik et al. 2021). Treatment of oak and walnut logs with bark attached is probably responsible for lack of sapwood moisture loss in the oak and the slight gain in the walnut logs subjected to VS compared with the slightly greater loss found in the VS-treated, debarked ohia logs.

The differences in time needed for treatment of three similar-diameter logs per treatment load for either the 56°C/30 min or 60°C/60 min schedule were attributed to differences in schedule temperature and holding time, although differences in log diameters used for a trial may have contributed to longer times. Although treatment times would logically be longer for treating more logs and longer logs in an operational run, VS is still a fast treatment compared with heat treatment in a drying kiln. Although phytosanitary treatment of dimension lumber and pallets via kiln-heating is recognized and accepted for global trade, its use for phytosanitary treatment of round wood has not been well studied. In comparison, VS has been shown to be an effective and efficient means of heating both rectangular sections of wood and round wood (Simpson 2001). Energy consumption for ohia log treatments would be an important consideration because energy costs in Hawaii are among the highest in the United States. For the current study, the authors calculated the cost per kilogram of log weight for the small-diameter logs (24 to 33 cm) in loads for tests 8 and 9 of the 56°C/30 min treatment to be $0.016 and $0.012, respectively.

Ohia logs in Hawaii are sold "skinned" (debarked) and "green" (not dried to a low moisture content). To date, the processed logs have been shipped at varying moisture contents that are correlated with the length of time logs air dry while stored in the mill yard before sale and shipping. Thus, VS-treated logs would be acceptable.

Superficial cracks or splits along the length and end checking of superficial depth of ohia logs are acceptable for traditional uses (e.g., decorative beams, porch railing systems). However, large logs with deep cracks and splits would not be suitable for structural supports of houses and load-bearing beams. In the current study, the effect of VS on ohia log quality was evaluated only 2 days after treatment, and minimal damage was documented. Future tests could include quality evaluation of VS-treated logs after several weeks or months of natural air drying in mill yards (i.e., the range of time logs are normally stored before shipping). Minimal end checking and splits of large logs of five different hardwood species were found on the same day treatment ended in earlier VS trials (Chen et al. 2017). We hypothesize that VS process would be an effective phytosanitary treatment that is energy and time efficient and would have no deleterious effects on quality of all sizes of ohia logs.

Recolonization of treated ohia logs was considered in this study because such logs might need to be held for several weeks or months depending on the terms of a log sale and other factors. Such logs would probably be held in the mill yard, where inoculum could be present in freshly cut logs coming directly from harvest sites in ohia forests. If phytosanitary treatment is conducted at the mill yard, the mill owner needs to know whether special precautions are needed to reduce potential for reinfestation by the ROD *Ceratocystis* species. Reinfestation experiments were conducted on *J. nigra* logs and natural wane lumber with different phytosanitation treatments in Tennessee (Audley et al. 2016). *Pityophthorus juglandis* (walnut twig beetle), the insect pest and primary vector of the thousand cankers disease pathogen (*G. morbida*), was found to recolonize the tested products. Thus, efforts to exclude insects from phytosanitized bark on walnut wood products would be needed for commercial trade. *Ceratocystis huliohia* and *C. lukuohia* were unable to colonize VS-treated ohia logs in this study. It is possible that treatment and subsequent air drying reduced the outer moisture content below a threshold that could not support superficial *Ceratocystis* growth (Tainter et al. 1984) or that the pathogens have limited saprophytic capacity to colonize nonliving tissues. Based on our findings of failure to recolonize, we hypothesize that exclusion efforts needed for heat-treated walnut logs would not be needed for VS-treated, debarked ohia logs.

Many practical matters should be considered before the VS process is adopted and used for ohia log treatments in Hawaii. Regulatory officials with the Hawaii Department of Agriculture evaluate proposed phytosanitary treatments for various commodities, including wood products. If approved, mill owners may consider the capital investment required for the needed equipment and extent of use for the equipment (i.e., they may conduct a cost–benefit analysis). Currently, only one mill on Hawaii Island has a vacuum chamber suitable for use in the VS process. Besides using VS for logs destined for off-island sales, the treatment could be considered for safe use of wood products from dying and recently killed ohia (i.e., salvage harvesting). Hundreds of thousands of ohia have been killed by ROD on Hawaii Island (College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa 2021) and not used because of concerns about spreading the pathogens to other areas of the island and potential for increasing intensity of the disease in other areas. Salvage harvest of ROD-killed ohia and phytosanitary treatment of cut logs could allow safe use of ohia wood from such trees. The forest industry in Hawaii is currently working toward increasing processing capacity and overcoming barriers for use of invasive tree species in an effort to manage them.

In summary, VS (either 56°C for 30 min or 60°C for 60 min) was found to be an effective and efficient method for killing viable *Ceratocystis* species associated with rapid ohia death in initial tests with short-length (about 2 m), large-diameter logs. Because a wide size range of ohia logs are produced by mills in Hawaii, further tests are needed to determine the range of treatment costs and total times required for different dimension products.

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