Borate and Quaternary Ammonia Dip Diffusion to Treat Fungal Pathogens of *Metrosideros polymorpha* Wood

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Abstract

Rapid Ohia Death is a major concern for the viability of ohia (*Metrosideros polymorpha)* in Hawaii and has led to restrictions on log movement. The potential for using disodium octaborate tetrahydrate (DOT) and didecyl dimethyl ammonium chloride (DDAC) dip diffusion treatments to control the two causal fungi (*Ceratocystis lukuohia* and *Ceratocystis huliohia*) was investigated. A 10 percent boric acid equivalent dip diffusion treatment killed the pathogens in 0.5-cm-thick disks obtained from 4.0- to 5.0-cm-diameter limbs of naturally colonized trees. DOT tended to diffuse more consistently in 50- to 60-cm-long bolts of small (4.0 to 9.0 cm) and large (9.1 to 17.0 cm) diameter healthy ohia compared with those bolts naturally infected by *C. lukuohia*. Diffusion periods longer than 6 weeks resulted in deeper penetration. Immersion (24 h) of logs (1.3 m long; 9 to 17 cm diameter) from *C. lukuohia* artificially inoculated trees in two forest locations in a 15 percent DOT/1 percent DDAC solution and storage for 10 weeks before evaluation resulted in incomplete elimination of the pathogen and lower boron concentrations in the inner sapwood than outer. Further investigations are needed to explore using either higher boron concentrations or longer diffusion periods to deliver fungicidal concentrations of boron deeper within the wood matrix.

The Hawaiian keystone tree ohia (*Metrosideros polymorpha* Gaudich.) is under attack by two newly discovered fungal pathogens, *Ceratocystis lukuohia* I. Barnes, T.C. Harrin, and L.M. Keith and *Ceratocystis huliohia* I. Barnes, T.C. Harrin, and L.M. Keith (Barnes et al. 2018). Together, these pathogens cause mortality commonly referred to as Rapid Ohia Death (ROD). This disease is quickly spreading across the state, causing ecological concerns for critical native habitat and dependent flora and fauna (Camp et al. 2019, Fortini et al. 2019). Both fungi colonize xylem and can disrupt water transport within trees, resulting in wilt, crown dieback, and death. The more aggressive *C. lukuohia* causes a systemic vascular wilt disease (Barnes et al. 2018, Hughes et al 2020).

The wood of ohia is prized for its use in strip flooring and as decorative poles and posts in Hawaiian-style homes and architecture (Skolmen 1974). Additionally, the wood is commonly used for firewood, smoking food, and traditional Hawaiian wood-working. Beyond its functional utility, ohia trees and its blooms represent a part of Hawaii's cultural story (Friday and Herbert 2006). Since the advent of ROD, the movement of infested wood is discouraged and a state interisland quarantine regulates the transport of ohia wood and plant material (Hawaii Department of Agriculture Amendment to chapter 4-72, amendment §4-72-13). Shipment of certified ''pathogen-free'' logs is permitted, but only for those that yield a negative result following laboratory testing of wood from each log for presence of either fungus. This testing process is time consuming and creates hardships for the mills that process and sell the logs. An effective log treatment that meets state phytosanitary

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standards would eliminate the need for such testing. An ideal treatment would be one that is simple to apply, requires minimal capital investment, is highly effective in killing the fungi, is safe to nontarget organisms, and is low cost. Identifying a suitable treatment presents some challenges, since the fungal species can colonize sapwood several centimeters or more inward from the surface, thereby rendering application of surface coatings ineffective. Fumigation, while potentially effective, would be far too costly for the small businesses involved and would pose safety and logistical challenges. Heat treatments are possible, but associated energy costs may be prohibitive.

One attractive mitigation strategy for this material would be boron dip diffusion treatment whereby logs are briefly dipped in a concentrated solution of boron and solid piled to allow the boron to diffuse into the wood. Dip diffusion using borates has offered protection against decay-fungi, termites, and other wood-boring insects (Harrow 1952, Anonymous 1972, McQuire and Gouldje 1972, Bunn, 1974, Grace et al. 1992, Freitag and Morrell 2005). The chemical has low mammalian toxicity, and the process is relatively simple and does not require specialized equipment (Jorge et al. 2004). However, this strategy has not yet been explored for either of the two ROD fungi or its ability to penetrate ohia wood. The goal of this work is to test the ability of dip diffusion using boron as disodium octaborate tetrahydrate (DOT) and quaternary ammonia as didecyl dimethyl ammonium chloride (DDAC) to diffuse into ohia logs and kill the fungal pathogens in diseased wood.

Materials and Methods

ROD-infected *M. polymorpha* wood bioassay after direct 24-hour immersion in DOT

Trees that exhibited symptoms of ROD were selected from the Hilo (HI) area, and the main stem tested for the presence of either *C. lukuohia* or *C. huliohia* DNA by diagnostic qPCR using previously described procedures (Heller and Keith 2018). One or two trees infected by each pathogen were felled by chainsaw and upper limbs cut into 1.5-m-long sections (= bolts). The bolts were end-sealed with a pruning sealant (Spectracide Pruning Seal, Spectrum Brands Inc., Madison, Wisconsin), wrapped in a plastic tarp to minimize drying, and stored in separate piles by colonizing pathogen at 5°C until processed. To identify which bolts contained the pathogen, each was rescreened by qPCR before further processing. PCR-positive bolts were debarked with a draw knife, and areas with visible xylem symptoms (vascular staining) were cut into circular disks (0.5 cm thick, 4.0 to 5.0 cm diameter) with a bandsaw, randomized, and numbered with a permanent marker (Fig. 1A). In total, 280 and 231 disks were able to be obtained from colonized bolts for the *C. lukuohia* and *C. huliohia* experiments, respectively.

The disks were immersed for 24 hours in treatment solutions containing 5, 10, or 15 percent DOT in water with or without 1 percent DDAC. The DDAC was added to provide an additional barrier against either fungal ingress or egress and to minimize mold growth. Water-immersed disks were used as controls. Each treatment was applied to 40 disks containing *C. lukuohia* and 33 disks containing *C. huliohia*. After soaking, disks were removed from the treatment solution, laid onto absorbent lab cloth (Versi-Dry, Thermo-Fisher Scientific, Waltham, Massachusetts) in a biosafety cabinet, and allowed to dry for 1 hour. Posttreatment fungal viability in the wood disks was evaluated using a carrot baiting technique (Moller and DeVay 1968). In brief, carrots were surface sterilized by wiping with 70 percent ethanol, peeled and cut into circular slices. Wood disks were placed between two carrot pieces, wrapped in wax film (Parafilm, Bemis Company, Inc, Neenah, Wisconsin), and individually stored in plastic bags (Fig. 1B). The baited disks were incubated at room temperature for up to 30 days. Baits were rated as positive when greyish *Ceratocystis* mycelia with characteristic fruiting structures (perithecia) were observed under a dissection microscope (Fig. 1C). Carrot pieces where mycelia were present but no fruiting structures visible (due to bacterial contamination) were assayed by diagnostic qPCR (Heller and Keith 2018). Experiments for *C. lukuohia* and *C. huliohia* were performed once, separately.

Ability of DOT to penetrate *M. polymorpha* at different storage time periods

Bolts (50 to 60 cm long; 4 to 17 cm diameter) were collected from the main stems of trees that were either healthy or naturally infected with *C. lukuohia* in April and September 2019 (Fig. 2). Since *C. lukuohia* is the dominant pathogen causing ohia mortality and there was a lack of sufficiently *C. huliohia*–colonized wood, we focused subsequent experiments solely on *C. lukuohia*–infected wood. Each bolt was debarked with a draw knife and labeled, and a 20- to 30-mm-thick disk was removed, weighed, oven-dried, and then reweighed to determine initial moisture content (MC). Bolts were sorted into ''small'' (4.0 to 9.0 cm) and ''large'' (9.1 to 17.0 cm) groups based on large-end stem diameter. Cut ends were sealed with latex paint to retard end-drying and allowed to dry for 1 day. The bolts were immersed for 24 hours in an excess of a solution containing either 10 or 15 percent DOT. Dip diffusion is normally performed for much shorter time periods (3 to 5 min), but ohia log processing is typically not rapid, and the authors thought that the longer dipping time might produce more uniform chemical uptake. After dipping, the samples were allowed to drain, then placed on timbers under cover, but with air flow for 6, 8, or 10 weeks to allow diffusion to progress. In each size category (small, large), four healthy and four diseased bolts were used for each of the three diffusion periods (6, 8, 10 wk) and two DOT (10%,15%) treatment combinations (48 treated bolts per size group). An additional four healthy bolts served as nonboron, water controls in each size category (104 bolts total).

After the diffusion period, 30-mm-thick disks were cut from each end of the sections and discarded. Additional 30-mm-thick disks were then cut from each end, weighed, oven-dried (104°C), and weighed to determine final MC. Two 30-mm-thick disks were cut from each end, and a single 30-mm-thick disk was cut from the center for qualitative boron assessment. The samples were air-dried overnight, then lightly sanded to create a clean surface before being assessed for boron penetration using turmeric according to procedures described in American Wood Protection Association (AWPA) Standard A68-16 (2020). The sanding reduced the risk that the saw cut had inadvertently spread boron across the surface. The depth of radial penetration was measured at four points around each stem beginning at the location with the deepest

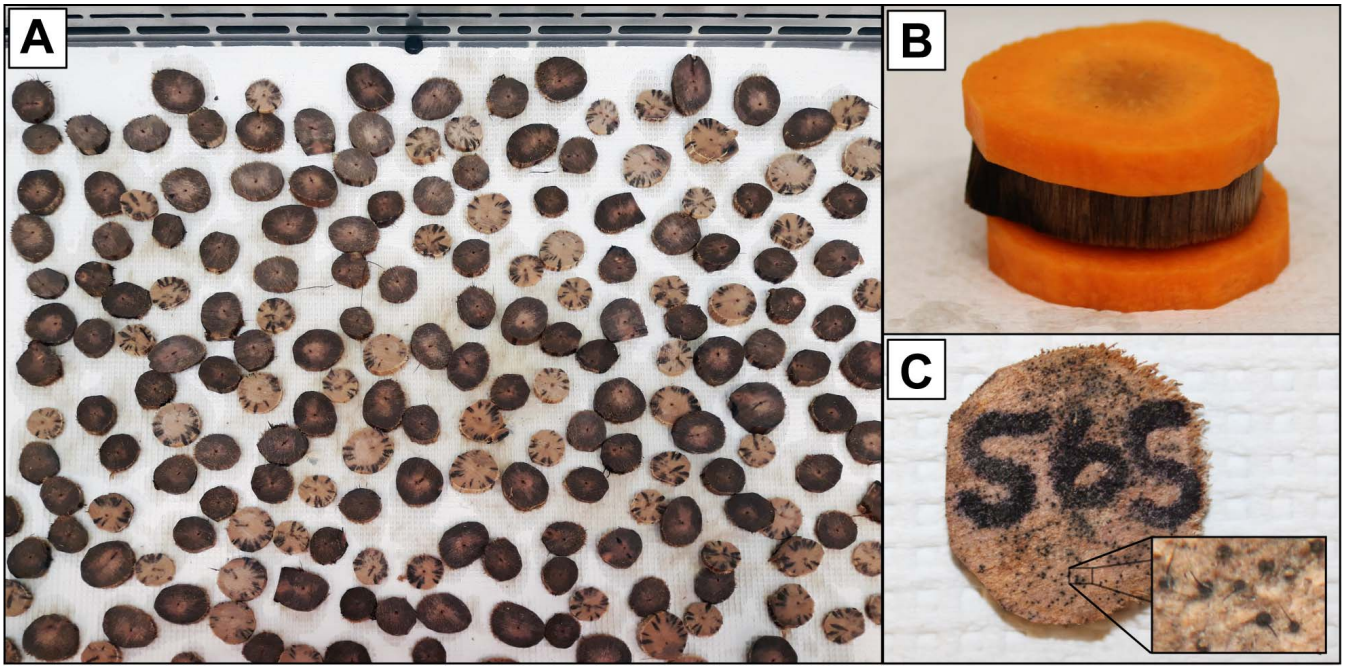

Figure 1.—ROD-infected M. polymorpha *wood bioassay. (A) Small wood disks (0.5 cm thick) prior to 24 hour DOT immersion. Brown wood discoloration and streaks indicates* C. lukuohia *colonization, (B) carrot bait to assess posttreatment fungal survival, and (C) minute black spots on wood disk denote fungal fruiting structures (perithecia in inset). Fungal growth on carrot or wood tissue indicate fungal viability.*

penetration and moving 90 degrees around the section. The samples were also photographed with a Canon EOS Rebel T7 camera (Canon, Melville, New York), and the percentage of stained area was calculated using Adobe Photoshop (Adobe, San Jose, California) software (Fig. 3A,B).

The central disk from each bolt was oven-dried at 65°C for 72 hours to kill any fungi present and shipped to the Oregon State University Biodeterioration Lab for boron analysis according to the azomethine-H method as described in AWPA Standard A65-15 (AWPA 2020). Boron content (as kilograms of boric acid equivalent per m^3) was determined in the outer sapwood (up to 25% radial depth) of all disks, while a second zone representing 50 to 75 percent of the radial depth was also analyzed in the larger (9 to 17 cm) diameter disks (Fig. 3C,D).

Figure 2.—Healthy (left) and C. lukuohia*–infested (right) wood from DOT penetration assays. The dark staining on the diseased bolt is associated with* C. lukuohia *colonization.*

Assay for fungus viability in logs

Based on the results of the previous trials, we selected a 15 percent DOT and 1 percent DDAC concentration to assess their ability to inhibit *C. lukuohia* growth in *M. polymorpha* logs. Trees were artificially inoculated using procedures described in Hughes et al. (2020). Inoculations occurred in June and September 2019 at Keaukaha Military Reservation (KMR) and Waiakea Forest Reserve (WFR), respectively. The trees were not felled until approximately 4 or 8 months after inoculation at WFR and KMR, respectively; thus allowing time for fungal colonization of the stems. Logs (9.0 to 17.0 cm diameter) were cut to 1.3-m lengths. Prior to treatment, two 30-mm-thick disks were cut 60 mm inward from each end and evaluated for *C. lukuohia* viability by aseptically chiseling small wood chips from 0.5 to 2.0 cm (outer zone) and 2.5 to 4.0 cm (inner zone) inward from the surface at four locations 90 degrees apart (eight baits per disk, 16 per log). Chips were baited between two carrot discs and were incubated and rated for *Ceratocystis* growth as described above. Additionally, when disks were initially cut to assess fungal viability, an adjacent 30-mm-thick disk was removed to assess initial wood moisture content. Log ends were sealed with latex paint to reduce end-grain penetration of treatment solution. Logs were submerged in either water or a 15 percent DOT/1 percent DDAC solution for 24 hours. A 24-hour immersion period was chosen because longer times did not pose a logistics problem, since this was not a high-production process, and we felt that longer time might result in more uniform uptakes. Eight logs were soaked in the DOT/DDAC solution and four in water alone from each field site (KMR and WMR). After the submersion period, logs were placed onto landscape timbers under a covered roof in ambient conditions for a 10-week diffusion period. After the

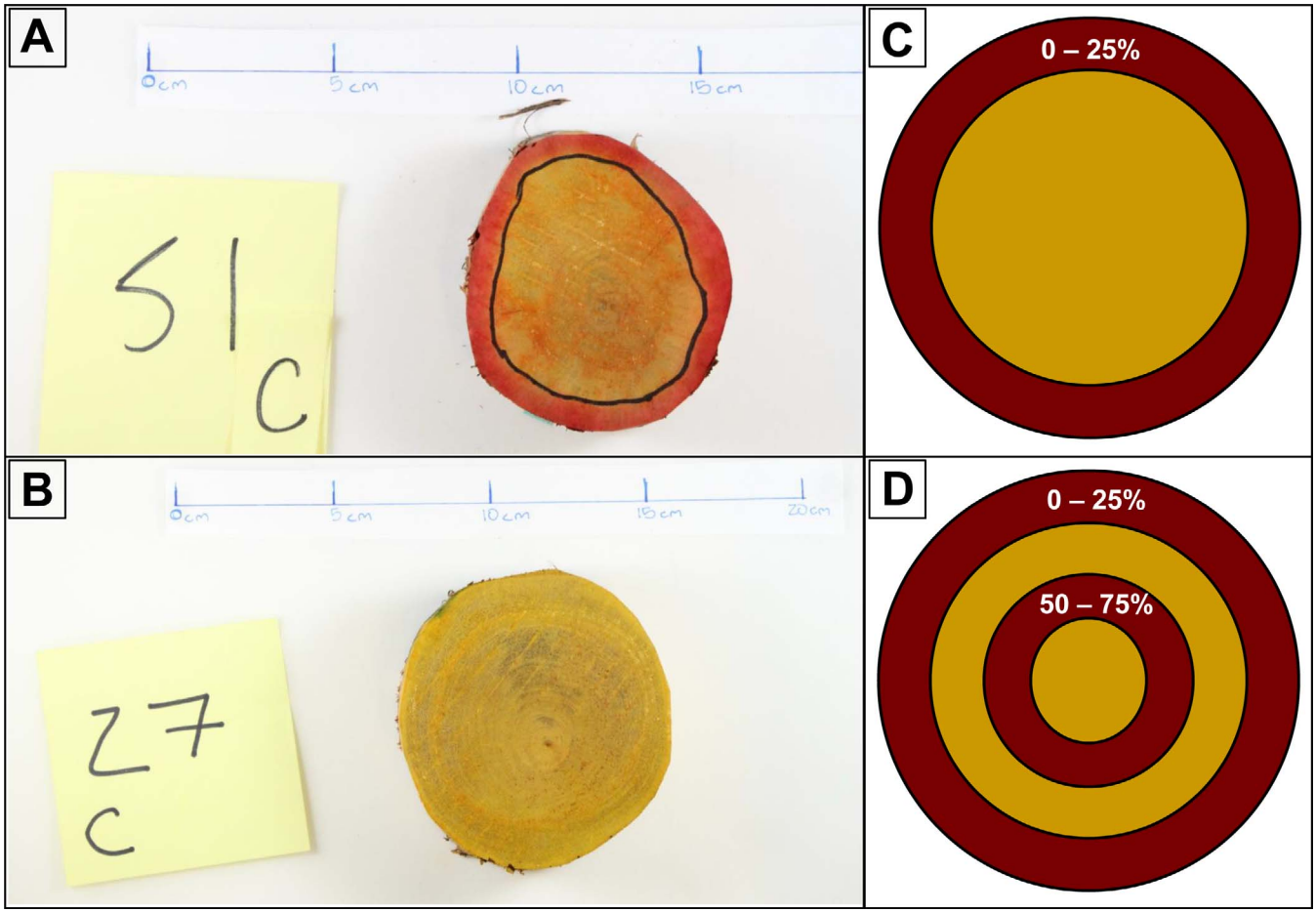

Figure 3.—Examples of visualization of DOT penetration on (A) a boron treated sample and (B) an untreated control as shown using a turmeric indicator solution, and sampling depths used to assess boron retentions for the (C) small (4.0 to 9.0 cm) and (D) large (9.1 to 17.0 cm) diameter samples.

diffusion period, the outer 30 cm was removed from each end and discarded to minimize the possible influence of end-drying. Two disks (30 mm thick) were cut from each end (four per log total) and refrigerated at 5°C. One disc from each end was used for posttreatment viability testing/carrot baiting, one was used to assess moisture content by oven-drying as described earlier, and the last was used for quantitative boron lab analysis. Boron analysis was conducted as described above, except that only half of the water-dipped control samples were analyzed.

Data Analysis

Data were analyzed using the ''Mixed Procedure'' in SAS 9.4 software (SAS Institute Inc., Cary, North Carolina). Visual ratings of radial DOT penetration depth (cm) and stained area (%) as shown by turmeric indicator dye were analyzed with DOT percentage (10% or 15%), diffusion period (6, 8, 10 wk), and presence/absence of *C. lukuohia* and their interactions as fixed effects. Boric acid equivalent (BAE) levels were calculated as above, except for large diameter disks where sampling depth (0 to 25% and 50 to 75% to core) and its interactions were added to the model. Sampling depth within each disk was added as a random effect to account for repeated measures per disk. The effects of bolt diameter size class (small or large) on boron uptake were examined by incorporating diameter measurements into the model as a fixed effect. Predip moisture content was a covariate, and control bolts (0% DOT) were omitted. Response variables were natural log transformed based on results of residual analyses, and selected means and confidence intervals were calculated. For the log viability assays, analyses were separated by location using a split plot design model. The fixed effects were bait position (inner vs. outer), treatment (DOT/DDAC vs. water control), and their interaction, with log-within-position as the random effect. The responses were transformed by square roots based on results of residual analyses. To compare the relationship between boron uptake (as kg/m^3 BAE) and baiting success, Kendall's and Pearson's correlations were calculated. For all experiments, the Type 3 Tests of fixed effects were run at $\alpha = 0.05$ and means and confidence intervals presented are given in the back-transformed scale (i.e., from logs or square roots).

Results and Discussion

ROD-infected *M. polymorpha* wood bioassay after direct 24-hour immersion in DOT

Recovery of *C. lukuohia* from the water-soaked, negative control baits was higher (92.5%) than for *C. huliohia* (54.5%). Neither fungus was recovered from the majority of DOT and DDAC treatments, except the 5 percent DOT

treatment of *C. lukuohia* in which 7.5 percent of baits yielded the fungus. The results indicated that a 10 percent DOT dip would deliver a sufficient amount of boron to kill the two pathogens in wood.

DOT penetration assays

The length of diffusion period (6, 8, 10 wk) and presence of *C. lukuohia* both significantly affected boron penetration as visualized by the turmeric indicator ($P = 0.003$ and $P = 0.037$, respectively), with periods longer than 6 weeks and absence of fungus increasing the degree of penetration (Table 1). The reduced DOT penetration by *C. lukuohia*–infected wood may have been due to xylem occlusion by a host defense response to limit internal fungus spread. Formation of tyloses, gums, and gels that block xylem flow is commonly associated with similar systemic vascular wilts of hardwood trees like laurel wilt and Dutch elm disease or in *C. fimbriata*–induced diseases (Et-Touil et al. 2011, Inch et al. 2012, Araujo et al. 2014, De Micco et al. 2016). Supporting this idea, Damayanti et al. (2020) found the presence of tyloses in *Macaranga conifera* wood negatively impacted boron permeability compared with a non–tylose-producing species. DOT concentration (10% or 15%) did not affect boron penetration ($P = 0.83$). The disk area stained by the turmeric indicator did not differ significantly among treatment effects ($P = 0.23$ to 0.87) and ranged from 49.0 to 58.4 percent. No staining by the indicator was visible in water-soaked controls.

DOT concentration, diffusion period, and fungal colonization treatment effects did not significantly differ among the treatments for the small diameter disks ($P = 0.06$ to 0.39) with retentions ranging from 3.34 to 17.28 kg/m^3 (BAE) among treatment combinations. Radial depth of the wood (0 to 25% vs. 50 to 75% to disk core) and presence of *C. lukuohia* were significant ($P \leq 0.001$) for the larger disks (Table 2). However, the interaction ($P = 0.009$) between fungal colonization and sampling depth was apparent, since very little boron was detected in the deeper bands (50 to 75% radial depth) sampled, regardless of the presence of *C. lukuohia* (Table 2). When the BAE level for the 0 to 25 percent depth for large and small diameter disks was analyzed with disk diameter and *C. lukuohia* presence/absence as factors, both smaller disks ($P \leq 0.001$) and those free of the fungal pathogen ($P = 0.01$) absorbed more boron than larger and infested disks (Table 3). Wood from the April samples had a predip moisture content of 60 to 63 percent (ovendry weight basis) and a final content of 23 to 30 percent, depending on the length of diffusion period. Moisture content in the September samples was slightly higher at 67 to 72 percent, with final levels of 29 to 32 percent after the diffusion period (Table 4). These results would suggest that conditions had declined to the point where there was far less free water available for diffusion at the end of the test, although it is important to note that the moisture content is the average of the entire cross section and may be higher farther away from the surface (Smith and Williams 1969).

Table 1.—Effect of diffusion period and the presence of C. lukuohia *on average depth of boron penetration in ohia logs. Treatments were considered significant at an $\alpha \leq 0.05$ using a Type 3 Test of fixed effects and 95 percent confidence intervals shown.*

Treatment effects[a]	Mean boron penetration (cm)	Confidence interval (cm)
Diffusion period (wk)**		
6	1.0	0.9–1.2
8	1.4	1.2–1.6
10	1.3	1.2–1.5
Fungus colonized*		
No	1.4	1.2–1.5
Yes	1.1	1.0–1.3

[a] $*P \leq 0.05$, $**P \leq 0.01$.

Table 2.—Boron retentions in ohia logs (9.1 to 17.0 cm diameter) with and without C. lukuohia *measured 10 weeks after DOT/DDAC dip treatment. Treatments and their interaction were considered significant at an $\alpha \leq 0.05$ using a Type 3 Test of fixed effects and 95 percent confidence intervals shown.*

Treatment effects[a]				
Radial depth sampled (R) (%)***	Fungal colonized (F)***	F × R Interaction**	Mean estimate (BAE (kg/m^3)	Confidence interval (BAE kg/m^3)
0–25	No		5.6	4.1–7.5
50–75	No		0.5	0.3–0.6
0–25	Yes		1.8	1.3–2.4
50–75	Yes		0.3	0.2–0.4

[a] $**P \leq 0.01$, $***P \leq 0.001$.

Assay for fungus viability in logs

Mean estimates of pretreatment fungal isolation rates in logs from the KMR site ranged from 35 to 53 percent for the outer and inner sapwood baits and were statistically similar before being treated ($P = 0.33$ to 0.98) (Tables 5, 6). The rate of *C. lukuohia* recovery from logs immersed in DOT/DDAC declined up to 97 percent over the 10-week diffusion period compared with their preimmersion carrot baits. Fungal viability of water-soaked controls from KMR declined 83 percent in the outer disk zone and 69 percent in the inner zone over the same 10-week diffusion period, suggesting that air-drying alone was associated with reduced fungal viability (Table 5). Although, the DOT/DDAC treatment did dramatically reduce *C. lukuohia* viability, the treatment effects of the boron dip were slightly above our cutoff for statistical significance ($P = 0.059$) (Table 6). Fungal isolation rates for all logs from the WFR site tightly

Table 3.—Boron retentions in ohia log sections of two different diameters with or without prior fungal colonization. Data analyzed were from the outer portion of the disk (0 to 25% radial depth to core). Treatments were considered significant at an $\alpha \leq 0.05$ using a Type 3 Test of fixed effects and 95 percent confidence intervals shown.

Treatment effects[a]	Mean (BAE kg/m^3)	Confidence interval (BAE kg/m^3)
Disk diameter (cm)***		
4.0–9.0	7.5	6.8–8.2
9.1–17.0	1.1	0.8–1.4
Fungus colonized (both diameter sizes)**		
No	3.4	2.8–4.2
Yes	2.3	1.9–2.9

[a] $**P \leq 0.01$, $***P \leq 0.001$.

Table 4.—Changes in bolt moisture content over a 6- to 10-week storage period. Data shown for small and large diameter bolts combined (n = 96 total). Water-dipped controls were omitted. For C. lukuohia *viability assays, per site: DOT/DDAC (n = 8) and water controls (n = 4).*

Experiment or site treatment	Diffusion period (wk)	Moisture content (%)		
		Initial	Final	Difference (initial − final)
Boron penetration assay				
April 2019 (n = 20/time)	6	59.7 ± 1.6	29.7 ± 1.5	30.0 ± 2.4
	8	62.0 ± 1.7	24.7 ± 1.3	37.3 ± 2.4
	10	63.0 ± 1.7	22.5 ± 1.1	40.8 ± 2.2
September 2019 (n = 12/time)	6	71.7 ± 2.0	31.8 ± 2.8	39.9 ± 3.5
	8	66.9 ± 1.6	29.3 ± 2.9	37.7 ± 2.5
	10	70.6 ± 2.2	28.7 ± 2.9	41.9 ± 2.9
C. lukuohia viability in logs				
KMR, DOT/DDAC	10	59.6 ± 3.7	35.2 ± 1.7	24.4 ± 4.5
KMR, water control	10	58.9 ± 4.6	31.4 ± 0.9	27.5 ± 3.7
WFR, DOT/DDAC	10	65.4 ± 1.2	33.5 ± 2.0	31.9 ± 1.8
WFR, water control	10	68.6 ± 1.1	31.6 ± 2.1	37.0 ± 2.2

Table 5.—Recovery of C. lukuohia *before (pre) and 10 weeks after (post) dip diffusion with a 15 percent DOT/1 percent DDAC solution.*

Site[a]	Treatment	Logs (n)	No. of assays	Mean fungal recovery (%)			
				Outer zone (5–20 mm)		Inner zone (25–40 mm)	
				Pre	Post	Pre	Post
KMR	DOT/DDAC	8	64	44.1 (29.4–61.8)	2.3 (0.0–9.6)	42.5 (28.1–59.9)	1.1 (0.3–7.0)
	Water control	4	32	52.6 (30.5–80.6)	9.1 (0.6–27.7)	35.4 (17.9–58.9)	10.9 (1.1–30.7)
WFR	DOT/DDAC	8	64	67.2 (52.0–84.4)	6.6 (0.3–21.2)	66.9 (51.7–84.1)	6.7 (0.3–21.4)
	Water control	4	32	68.0 (46.9–93.0)	60.5 (24.0–113.4)	67.2 (46.3–92.1)	40.5 (12.2–85.4)

[a] KMR and WFR are the Keaukaha Military Reservation and Waiakea Forest Reserve sites in Hilo, Hawaii. 95 percent confidence interval of the estimated means are provided in parentheses.

Table 6.—Results of Type 3 test of fixed effects for experiments testing the viability of C. lukuohia *in ohia logs immersed in 15 percent DOT/1 percent DDAC solution and subjected to a 10-week diffusion period.*

Site	Fixed effects	df	Mean fungal recovery (%)	
			Pretreatment P	Posttreatment P
KMR	Baiting location	1,20	0.3322	0.9321
	Dip treatment	1,20	0.9798	0.0586
	Interaction	1,20	0.4200	0.7024
WFR	Baiting location	1,20	0.9578	0.5660
	Dip treatment	1,20	0.9523	0.0012
	Interaction	1,20	0.9781	0.5574

grouped between 67 to 68 percent before the treatment soak and diffusion period (Table 5). The application of boron reduced fungal detection rates 90 percent compared with pretreatment isolations. The control treatment (water immersion) saw a 11 and 40 percent drop in fungal recovery after the diffusion period for the outer and inner baiting locations, respectively (Table 5). Treating logs in the DOT/DDAC solution had a significant effect on *C. lukuohia* viability at WFR compared with the control treatment (P = 0.001). The number of positive carrot baits was similar between the inner and outer sampling zones for both experiments (P = 0.932, 0.557) (Table 6). Mean boron retentions for DOT/DDAC–dipped samples were consistent between sites and were 0.33 and 0.57 kg/m^3 BAE for the inner and outer zones, respectively. Water-dipped controls from WFR had a mean of 0.28 kg/m^3 BAE between the two sampling zones, while control levels were slightly lower at KMR (0.18 kg/m^3 BAE, data not shown). Pearson's and Kendall's correlations between BAE levels and posttreatment rates of fungal detection were not significant (P = 0.28 and 0.33, respectively) (data not shown). Wood from KMR had a starting predip moisture content of around 60 percent and that from the WFR site ranged from 65 percent to 69 percent. By the end of the diffusion period, logs from both sites had a final moisture content of 31 to 35 percent (Table 4). The results indicate that, while conditions were initially suitable for boron diffusion, they declined to levels that would be marginal for further movement. However, that may be less important if sufficient movement occurred earlier in the diffusion process.

While the results indicate that boron treatment was associated with a decline in fungal recovery, the treatment could not completely eliminate the fungus from commercial-sized samples. The depths of boron penetration did not exceed the hyphal penetration depths recorded for ohia naturally infected by *C. lukuohia* (up to 4.0 cm, Hughes et al. 2020), suggesting insufficient penetration. As a result, boron dip diffusion, while showing promise, does not appear to be completely effective or suitable as a phytosanitary treatment for ohia without further refinements.

Possibilities for improving this process could include increasing the diffusion period to allow boron to diffuse more thoroughly through the wood, increasing the boron concentration, or potentially wrapping the logs to retain moisture and produce conditions more conducive to boron diffusion (Harrow 1952, McQuire and Gouldje 1972, Ra and Conners 2001, Lebow et al. 2013). Another option that might be explored is to assess boron/glycol treatments which purport to produce deeper diffusion into the wood.

Conclusions

Bioassays using fungus-colonized disks demonstrated that DOT ($\geq$10%) inhibited the growth of *C. lukuohia* and *C. huliohia* in a laboratory setting. Mean depth of DOT penetration in ohia bolts as visualized by turmeric indicator was less than 1.5 cm, and the boron retention declined with increased sampling depth. Fungus-free wood retained more DOT than wood infested with *C. lukuohia*. The reduced DOT penetration by *C. lukuohia*–infected wood was likely due to the production of tyloses, gums, and gels in xylem vessels in response to fungal attack, which is a common defense mechanism in hardwood trees against systemic vascular wilt pathogens. We hypothesize that these occluded wood vessels may have limited normal borate diffusion pathways. Trials of 1-m log sections using a 15 percent DOT solution and 10-week diffusion period greatly reduced, yet failed to completely eradicate, viable *C. lukuohia*. Future studies using stronger borate solutions with glycol and a longer diffusion period could potentially increase borate penetration and retention in ohia wood.

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