ORIGINAL ARTICLE

Screening of *Metrosideros polymorpha* (ʻōhiʻa) varieties for resistance to *Ceratocystis lukuohia*

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Abstract

Stands of the landscape-dominant tree, *Metrosideros polymorpha* (ʻōhiʻa) on the Island of Hawaiʻi, HI., USA, are dying from a phenomenon known as rapid ʻōhiʻa death (ROD). Approximately 180,000 acres of forest have already been impacted by the disease, the majority of which is attributed to *C. lukuohia*, the more aggressive of the two *Ceratocystis* species responsible for ROD. Three isolates of *C. lukuohia* were compared for differences in aggressiveness. Mean disease severity and mean number of days to death did not differ among the three isolates of *C. lukuohia*. A single isolate was selected for use in a resistance screening of 128 plants across four varieties of *M. polymorpha* occurring on Hawaiʻi Island: *incana*, *glaberrima*, *polymorpha* and *newellii*. Disease severity was lower in early-successional var. *incana* (38.84%) and riparian var. *newellii* (36.11%) compared to late-successional var. *glaberrima* (61.27%) and high-elevation var. *polymorpha* (70.27%). Var. *incana* and var. *newellii* also had the lowest mortality (63% and 77%, respectively) while var. *glaberrima* (86%) and var. *polymorpha* (100%) had the highest mortality. Eighty-five per cent of the plants that died did so within 49 days post-inoculation and mean number of days to death was significantly higher for var. *glaberrima* (46) compared to var. *polymorpha* (31). The results of this preliminary study suggest that some form of resistance might be present in natural populations of at least one variety of *M. polymorpha* on Hawaiʻi Island and should be investigated more intensively.

KEYWORDS
ceratocystis, disease type, metrosideros, wilt

1 | INTRODUCTION

Metrosideros polymorpha Gaud. (known in Hawaiian as ʻōhiʻa) is an endemic keystone species that dominates native Hawaiian forests and is the most abundant tree species in the islands. *M. polymorpha* ranges from sea level to 2,500 m in elevation and is vital for preserving watersheds and providing habitat for other native species (Friday & Herbert, 2006). This tree is also important to native Hawaiian culture, where different parts of the tree have been used for medicine, religious idols and household implements (Krauss, 2001; Malo, 1903). *M. polymorpha* is known for its

phenotypic diversity, comprising a large number of varieties or morphotypes that are distinguished chiefly through vegetative characters (Dawson & Stemmermann, 1990; Stacy & Sakishima, 2019). Variation among forms is heritable and not a result of phenotypic plasticity (Cordell et al., 1998; Stacy et al., 2016, 2020). On Hawaiʻi Island, there are five recognized varieties (vars.) of *M. polymorpha* according to Dawson and Stemmermann (1990): *incana*, *glaberrima*, *macrophylla*, *newellii* and *polymorpha*. Due to the lack of consistent defining features of var. *macrophylla* and its high genetic similarity with the widespread var. *glaberrima* (Stacy et al., 2014), here we do not treat var. *macrophylla* as a separate taxon. Habitat ranges

for var. *incana*, var. *glaberrima* and var. *polymorpha* are partitioned mainly by elevation and substrate age (Corn & Hiesey, 1973; Dawson & Stemmermann, 1990; Stemmermann, 1983), while var. *newellii* is restricted to riparian areas on the eastern part of the island (Dawson & Stemmermann, 1990; Ekar et al., 2019). Many differences have been observed among these varieties that appear to be associated with adaptation to different environments, including leaf chlorophyll concentrations (Martin & Asner, 2009), xylem vessel diameter (Fisher et al., 2007), shade tolerance (Morrison & Stacy, 2014) and tolerance to strong water current (Ekar et al., 2019).

Two newly described species of *Ceratocystis* were discovered to be the cause of widespread death of 'ōhi'a trees on Hawai'i Island (Barnes et al., 2018; Keith et al., 2015). Although the pathogens cause two different diseases (wilt and canker), the main symptoms that result from infection by either pathogen are a swift browning of the canopy and black staining of the sapwood. This phenomenon is collectively referred to as rapid 'ōhi'a death (ROD; Barnes et al., 2018; Keith et al., 2015). *C. lukuohia* I. Barnes, T.C. Harr. & L.M. Keith is more aggressive, spreads throughout the entire tree and kills it in as little as two months (Hughes et al., 2020). *C. huliohia* I. Barnes, T.C. Harr. & L.M. Keith forms discrete cankers around entry points, suggesting that multiple infections of *C. huliohia* may be required to kill a mature tree (Juzwik et al. unpublished). As of early 2020, approximately 180,000 acres of *M. polymorpha* forest have been affected by ROD (ROD SRP 2020). On Hawai'i Island, impacted areas range from low-elevation (245 m elevation), young-substrate (≤750 year-old substrate; Sherrod et al., 2007) locations such as Leilani Estates in Puna, to high-elevation (1,650 m elevation), old-substrate (approximately 150,000 year-old substrate; Sherrod et al., 2007) locations such as Kohala Volcano. Across impacted forests, ROD-associated mortality ranges from sporadic to greater than 90% (ROD SRP 2020). *C. lukuohia* is responsible for most of the widespread mortality, which is why it was the focus of the current study. Currently, *C. lukuohia* is on Hawai'i Island and Kaua'i (Heller et al., 2019; Keith et al., 2015). To date, there is no known cure for infected trees; therefore, preventative methods are necessary to keep the pathogens from spreading into pristine forests throughout the Hawaiian archipelago.

One of the primary methods of combatting forest tree diseases is the outplanting of disease-resistant stock or using mechanical and chemical tools to proactively prevent the spread of disease in vulnerable, unimpacted stands or reactively in highly impacted stands (Juzwik et al., 2011; Schoettle & Sniezko, 2007; Sniezko, 2006). Breeding trees for disease resistance is a challenge because it requires the existence and identification of resistant individuals in natural populations. Also, resistant individuals are useful only if they can avoid being overcome by the most aggressive strains of the pathogen. Thus, highly aggressive strains of the pathogen must be isolated, identified and used to test potentially resistant stock (Oliveira et al., 2016).

Through the screening of natural isolates, we identified a single, highly aggressive isolate of *C. lukuohia* and used it to screen seedlings and rooted cuttings of four varieties of *M. polymorpha* on Hawai'i Island for resistance. This work marks the first step towards identifying ROD-resistant material of this keystone tree species.

2 | MATERIALS AND METHODS

2.1 | Isolates

Ceratocystis lukuohia isolates were selected from a collection at the USDA ARS Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center (PBARC) in 2016. Three isolates (P14-1–1, P15-80 and P16-7) were selected for this study because they represented distinct disease clusters at the time. Isolate P14-1–1 was isolated in 2014 from Leilani Estates, which is known as the epicentre of the ROD outbreak in 2014 and an area of high disease incidence. Isolate P15-80 was isolated in 2015 from Fern Forest, an area with low disease incidence at that time, but is geographically close to Leilani Estates (approximately 22 km). Isolate P16-7 was isolated in 2016 from Kā'u, which was a new outbreak located more than 70 km away from Leilani Estates and Fern Forest.

2.2 | Isolate aggressiveness testing

Metrosideros polymorpha plants were grown from cuttings from a single mother tree known to be susceptible to the pathogen based on previous inoculation experiments (Keith et al. unpublished). While moderately disease-resistant plants would have been ideal for comparing aggressiveness, no such plants were available at the time of the experiment. The mother tree is a naturally occurring tree at PBARC that is believed to be a hybrid of *M. polymorpha* var. *glaberrima* and var. *incana* based on leaf characters (Stacy et al., 2016). Each cutting was dipped in a 1:5 dilution of a rooting solution (Dip'N Grow®, Clackamas, OR) and placed in 1 ½-inch (3.81 × 3.81 cm) rooting cubes (Oasis Grower Solutions, Kent, OH). Cuttings were then placed in a greenhouse and watered by mist every 30 min for 30 s. Once roots were apparent, cubes containing individual plants were placed in 4-inch (10.16 × 10.16 cm) pots with high drainage, soilless potting mix with perlite (Sunshine Mix #4® Sun Gro, Agawam, MA) as the medium, and fertilized (10 N: 5 P: 10 K) (Apex® NPK Max,; Simplot, Boise, ID) every 3 to 5 months. Plants were then switched to a watering schedule of twice per day for 8 min each.

Following Keith et al. (2015), 7-day-old cultures grown on 10% V8 agar were flooded with 2 ml of sterile water, scraped with a rubber policeman, and the resulting suspension was poured off into a sterile petri dish. The suspension was diluted down to 1×10^6 spores/ml using a hemocytometer, and sterile 6 mm filter paper discs (GE Healthcare, Marlborough, MA) were soaked in the suspension for 30 s. The discs were placed on sterile 10% V8 agar plates and allowed to dry until excess moisture had evaporated. The plates were then incubated at 25°C for two days under light. Using a sterile scalpel, *M. polymorpha* plants were inoculated roughly 2 cm above the soil by creating a tangential wound in the stem deep enough to reach the

xylem tissue. A single *C. lukuohia*-colonized filter paper disc (6 mm diameter) was inserted into the wound which was wrapped in laboratory film (Parafilm, Bemis Company, Inc., Neenah, WI) to secure it in place and keep the wound from desiccating. Following inoculation, plants were randomly placed in a growth chamber (Controlled Environments Ltd., Manitoba, Canada) set to 28°C with a 12-hr photoperiod. Four individual plants were inoculated per isolate of *C. lukuohia*, and two plants were inoculated with a filter paper disc soaked in sterile water, which served as a negative control. Plants were watered by hand every 2 days. The times (in days) to expression of external disease symptoms (leaf wilt) and plant death were recorded. When an individual died, the plant was removed from the growth chamber, and the bark was scraped away to reveal any xylem discoloration. The stem was vertically split to further observe internal symptoms. Measurements of xylem discoloration length and plant height were recorded and used to calculate disease severity, which is defined as the quotient of xylem discoloration length over plant height multiplied by 100 (Oliveira et al., 2016). A stem segment approximately 3 mm in length was removed from the inoculation site and from a stained segment of the main stem 3–5 cm away from the inoculation site depending on the height of the main stem. Slices of wood were shaved from these stem sections and placed on a slice of carrot to re-isolate the fungus (Moller & DeVay, 1968).

Re-isolated *C. lukuohia* was identified by morphology and qPCR using chunks of the fungus-colonized carrots (Heller & Keith, 2018) to confirm that the pathogen spread beyond the inoculation site and was the cause of the advanced internal discoloration and external symptom development. The inoculation experiment was conducted twice.

2.3 | Resistance screening

Four varieties of *M. polymorpha* that occur on Hawai'i Island were included in this experiment: *M. polymorpha* var. *incana*, var. *glaberrima*, var. *newellii* and var. *polymorpha*. Due to the limited availability of seed-derived plants, experimental plants of var. *incana*, var. *glaberrima* and var. *polymorpha* were supplemented with rooted cuttings. *M. polymorpha* var. *newellii* plants were grown only from seed.

Seedlings of all four varieties were obtained from the *Metrosideros* Research Greenhouse at the University of Hawai'i at Hilo's Pana'ewa Farm, Hilo, HI. Fruits derived from open-pollinated flowers were collected from multiple sites throughout east Hawai'i Island and seeds were sown in 2013. To minimize the influence of hybrids in the seedling populations, with a single exception, seeds were collected from pure-variety stands (where > 95% of all stems are of a single variety) of *M. polymorpha*. Because riparian var. *newellii* occurs in narrow populations embedded within var. *glaberrima*-dominated wet forest, seeds from var. *newellii* may be more likely to include hybrids. A minimum of five trees were sampled per site. Var. *newellii* seeds were collected from Pi'ihonua (335 m elevation); var. *polymorpha* seeds from Stainback Highway (1575 m elevation), Mauna Loa Strip Road (2050 m elevation) and Mauna Loa Access Road (2,300 m elevation);

var. *glaberrima* seeds from mile marker 12 on Saddle Road (853 m elevation); and var. *incana* seedlings from Saddle Road mile marker 12 (853 m elevation), Paradise Park (90 m elevation), South Puna (275 m elevation) and Ka'ū desert (950 m elevation). These seedlings were transported to PBARC, planted in 4-inch (10.16 × 10.16 cm) pots and grown following the methods used in section 2.2. Control plants were two-year-old seedlings derived open-pollinated flowers from the same suspected hybrid tree at PBARC that provided cuttings for the isolate aggressiveness testing.

Cuttings were obtained in 2015 from the *M. polymorpha* common garden at the Hawai'i Volcano Experimental Research Station (Volcano, HI). Common garden trees were grown from seeds derived from open-pollinated flowers and collected from populations at 107 m to 2,469 m on the eastern slope of Mauna Loa in 1991–1992 (Cordell et al., 1998). The garden includes trees that can be identified morphologically to variety (var. *incana*, var. *glaberrima*, or var. *polymorpha*) as well as apparent inter-varietal hybrids; cuttings were taken only from individuals that possessed phenotypes of pure varieties. Cuttings were stored in a cooler with ice and transported to PBARC for planting following the methods in section 2.2. One rooted cutting per mother tree was used for resistance screening.

Plants were inoculated following the methods described in section 2.2. The experiment was conducted twice using the same growth chamber (Figure S1). Each trial consisted of nine individuals of var. *newellii*; 21 individuals each of var. *incana*, var. *glaberrima* and var. *polymorpha*; and 2 positive control (i.e. PBARC suspected hybrid) plants. The latter plants were produced in the same manner as those used in Section 2.2. In each trial, two plants from each variety were inoculated with a filter paper disc soaked in sterile water and served as negative controls. The plants were randomly placed in a growth chamber set to 28°C with a 12-hr photoperiod and 90% humidity. Plants were watered every 2 days and monitored for 17 weeks (119 days), during which the number of days to death was recorded for each individual. Plants were removed from the growth chamber and dissected upon death. Xylem discoloration was measured, and approximately 1 mm thick slivers of stem wood 3–5 cm from the inoculation site were shaved from inoculated and negative-control plants to examine for the presence of aleurioconidia under a compound microscope. Wood shavings were also used for carrot baiting and qPCR to test for the presence of *C. lukuohia*. Plants that survived were moved to a greenhouse for future studies and, thus, were not dissected. Inoculated plants that died were excluded from analysis if carrot baiting and qPCR failed to detect *C. lukuohia* (2 var. *glaberrima*, 1 var. *polymorpha* and 1 var. *newellii*).

2.4 | Statistical analysis

For the isolate aggressiveness experiment, a two-way ANOVA with Tukey's HSD was conducted to assess differences in disease severity between isolates and experimental trials. The latter variable was included to account for any differences between experiments. A Mann–Whitney test was conducted to analyse the difference in

TABLE 1 Mean dimensions and mortality (%) of plants of four *Metrosideros polymorpha* varieties inoculated with *Ceratocystis lukuohia* P14-1–1 for 119 days

M. polymorpha variety	Number of plants		Mortality (%)	Mean stem diameter (mm)	Mean plant height (mm)	Mean disease severity (%)	Mean time to death (days)
	Total	Dead					
glaberrima	36	31	86	4.8 ± 0.3a	308.2 ± 15.6a	61.3 ± 4.0b	45.9 ± 3.9a
incana	38	24	63	4.7 ± 0.3ab	239.4 ± 10.2b	38.8 ± 5.1a	46.4 ± 6.1ab
newellii	13	10	77	3.4 ± 0.5bc	198.8 ± 26.9b	36.1 ± 11.0a	50.0 ± 14.1ab
polymorpha	37	37	100	3.5 ± 0.2c	233.2 ± 19.8b	70.3 ± 3.7b	32.5 ± 1.8b

Note: Mean stem diameter, mean plant height, mean disease severity and mean time to death are represented as mean ± *SEM*. Letters in each column represent significant differences based on a Tukey's or Games–Howell post hoc tests ($p < .05$).

mean number of days to death between trials, and a Kruskal–Wallis test was used to assess the differences in mean number of days to death among isolates. All statistical tests were conducted using R statistical software version 3.4.1 (R Core Team, 2017).

For the resistance screening experiment, mean disease severity and mean number of days to death were compared between plants propagated from cuttings or seed using Wilcoxon rank sum tests to observe the effect, if any, of propagation technique on plant resistance. The relationship of plant height and stem diameter on disease severity and time to death were analysed using Spearman's rank-order correlations (ρ). A two-way ANOVA and Tukey's HSD were conducted to analyse differences in disease severity by trial and ʻōhiʻa variety. Between-trial variation in the mean number of days to death was examined using a Wilcoxon rank sum test. The relationship between mean number of days to death and variety was analysed using Welch's ANOVA and a Games–Howell post hoc test to account for heteroscedasticity in the data. Variation in the number of dead and living plants among varieties was analysed using a chi-squared test. All statistical tests were conducted using the R base software version 3.4.1 (R Core Team, 2017). Welch's ANOVA and the Games–Howell post hoc tests were conducted using the 'userfriendlyscience' package version 0.6–1 (Peters, 2017). The percentages of surviving plants over the course of the resistance screening experiments were visualized using the 'ggplot2' package version 3.3.0 (Hadley et al., 2020) in R base software version 3.6.3 (R Core Team, 2020).

3 | RESULTS

3.1 | Isolate aggressiveness

All ramets of *M. polymorpha* that were inoculated with the 3 isolates of *C. lukuohia* died during the experiment, except for one plant inoculated with isolate P14-1–1. Seven ramets were dissected and assessed for each isolate. Succulent shoots of inoculated plants began to wilt 11–22 days post-inoculation. The entire plant wilted within 22–53 days post-inoculation and within 8–40 days after first observations of symptom development. All plants except one (inoculated with P15-80) contained black xylem discoloration and a degraded or absent pith. Discoloured stem tissue from inoculated plants examined at 400X had aleurioconidia embedded in the wood. Carrot

slices used to re-isolate the pathogen from the inoculated plants produced perithecia after approximately 7 days and all inoculated plants tested positive for *C. lukuohia* DNA through qPCR. All negative-control plants remained healthy for the duration of the experiment, and their tissue showed no visible staining or aleurioconidia.

Disease severity did not differ among isolates P14-1–1, P15-80 and P16-7 ($F = 2.61$, $p = .107$) or between trials 1 and 2 ($F = 1.47$, $p = .245$), and there was no observable interaction between isolate and trial ($F = 0.36$, $p = .743$). However, there was a significant difference in mean number of days to death between trials ($W = 12$, $p = .003$). Therefore, data from trials 1 and 2 were analysed separately. In both trials, mean number of days to death did not differ among the 3 isolates (trial 1: Kruskal–Wallis $\chi^2 = 0.05$, $p = .978$; trial 2: Kruskal–Wallis $\chi^2 = 0.03$, $p = .988$). Since the isolates were equally aggressive, *C. lukuohia* P14-1–1 was selected as the single isolate used for resistance screening because it had been previously used by Keith et al. (2015).

3.2 | Resistance screening

Rooted cuttings ranged from 62 to 639 mm in height (average ± standard error = 251 ± 18 mm) and 2.97 to 11.61 mm in diameter (5.64 ± 0.32 mm), and seedlings were 83 – 646 mm in height (245 ± 10 mm) and 1.43 – 11.26 mm in diameter (4.57 ± 0.18 mm).

Plants began wilting as early as 8 days post-inoculation, and some plants died as early as 12 days post-inoculation. All plants that died had black discoloration of stem tissue typical of *C. lukuohia* infection (Figure S2). There were no significant differences in any of the variables examined between experimental trials, so data from the two trials were analysed together. No differences in mean disease severity ($W = 1,196$, $p = .165$) or mean days to death ($W = 1,123$, $p = .405$) were observed between rooted cuttings and seedlings. Although mean disease severity did not correlate with plant height ($\rho = 0.040$, $p = .698$) or stem diameter ($\rho = -0.130$, $p = .192$), time to death was modestly, but significantly influenced by size (plant height: $\rho = 0.376$, $p < .001$; stem diameter: $\rho = 0.280$, $p = .004$). Overall, var. *glaberrima* individuals were taller than the other varieties on average ($F = 6.24$, $p < .01$; Table 1), and plants of var. *glaberrima* and var. *incana* had thicker stems compared to those of var. *newellii* and var. *polymorpha* ($F = 6.62$, $p < .01$; Table 1).

There were significant differences among the varieties for the three disease measurements recorded. Plant mortality differed among the varieties (χ^2 = 18.10, p < .001) with the largest contributors to the χ^2 value being var. *incana* (dead r_{std} = −3.701, alive r_{std} = 3.701) and var. *polymorpha* (dead r_{st} = 3.373, alive r_{std} = −3.373). Var. *glaberrima* (dead r_{std} = 0.718, alive r_{std} = −0.718) and var. *newellii* (dead r_{std} = −0.532, alive r_{std} = 0.532) contributed less. Mean time to death and mean disease severity differed among varieties as well (F = 4.45, p = .011 and F = 10.57, p < .001, respectively; Table 1). Var. *glaberrima* survived longer than var. *polymorpha* post-inoculation (p = .013), and time to death was similar for var. *incana* and var. *newellii*. Var. *incana* and var. *newellii* displayed greater resistance as compared to var. *glaberrima* and var. *polymorpha*. Eighty-five per cent of the mortality occurred within 49 days post-inoculation for all tested varieties (Figure 1). Five individuals of var. *glaberrima* (14%), three of var. *newellii* (23%) and 14 of var. *incana* (37%) survived *C. lukuohia* infection for the duration of the experiments.

4 | DISCUSSION

The results of this study suggest that the three isolates of *C. lukuohia* that were tested were equally aggressive towards the *M. polymorpha* ramets. This result is supported by genotypic analysis conducted by Barnes et al. (2018), where 59 *C. lukuohia* isolates were shown to be clonal at various loci. These lines of evidence further support the hypothesis that *C. lukuohia* is a clonal lineage and a recent introduction (Barnes et al., 2018).

Results from our resistance trials suggest that the four varieties of *M. polymorpha* on Hawai'i Island respond differently to infection by *C. lukuohia*, with var. *incana* showing the most resistance (lowest per cent mortality, disease severity) compared to var. *newelleii*, var. *glaberrima* and var. *polymorpha*. In addition, the number of days from inoculation to death was significantly higher for var. *glaberrima* than for var. *polymorpha*. While time to death for var. *incana* and var. *newellii* were statistically similar to both var. *glaberrima* and var. *polymorpha*, they had the highest mean values. This result is most likely due to the variation in the time to death for these varieties, as they also had the highest standard errors. Of the four varieties tested, var. *incana* had the highest survivorship, with 37% of plants surviving infection. Twenty-two plants, 18% of the 124 plants inoculated with *C. lukuohia*, survived the two 17-week inoculation experiments. In early 2020, more than two years post-inoculation, four var. *incana* and one var. *newellii* are still alive (11% and 8% of all *C. lukuohia*-inoculated var. *incana* and var. *newllii* plants, respectively). Mean disease severity and time to death were highly variable for var. *newellii* as compared to the three other varieties, which could be attributed to low sample size (n = 10). Therefore, more var. *newellii* plants will need to be tested to gain a better understanding of var. *newellii* susceptibility
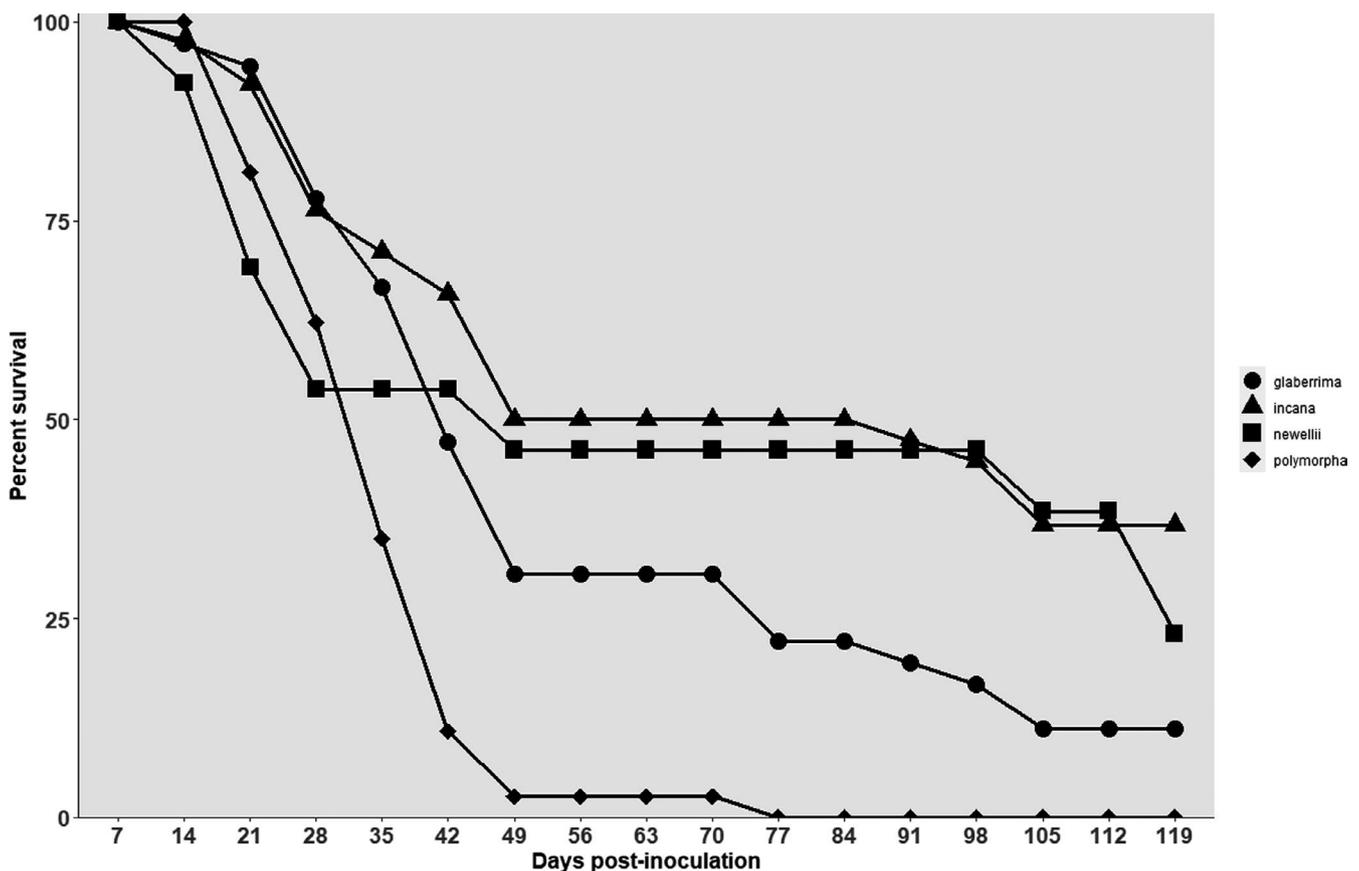

FIGURE 1 Percentage of surviving *Metrosideros polymorpha* var. *incana*, var. *newellii*, var. *glaberrima* and var. *polymorpha* over time following inoculation with *Ceratocystis lukuohia*

to *C. lukuohia*. Combining the results from all experiments, the data suggest that var. *incana* may be most resistant to *C. lukuohia* compared to the other varieties tested in this study. These results are similar to those seen in screenings of *Eucalyptus* and mango (*Mangifera indica* L.) with *Ceratocystis*, where cultivars varied in susceptibility to disease, but a few cultivars were identified as highly resistant (Oliveira et al., 2016; Zauza et al., 2004). Plants that survived the resistance screening experiments are being grown to propagate more cuttings for additional screening. While the term 'resistant' is used to describe survivors of *C. lukuohia* infection, it is not clear whether the plants are truly resistant to disease or merely tolerant to infection. Future experiments will need to be conducted to better understand the mechanisms underlying this potential resistance.

Genetic diversity of a host population is important for buffering the effects of an emerging disease because there is a greater chance that disease resistance will be naturally present in the population compared to a less diverse population (Altizer et al., 2003; King & Lively, 2012). The substantial genetic diversity of the landscape-dominant tree, *M. polymorpha*, which comprises four ecologically diverged varieties on Hawai'i Island, should provide an advantage for the species in the face of ROD. The varieties of *M. polymorpha* included in this study are partitioned across environmental gradients or ecotones marked by striking changes in abiotic factors, including temperature, light, nutrient availability and mechanical stress (Corn & Hiesey, 1973; Drake & Mueller-Dombois, 1993; Ekar et al., 2019; Pearson & Vitousek, 2002; Stemmermann, 1983). Disruptive selection imposed by contrasting environments has resulted in numerous physiological and morphological adaptations in *M. polymorpha* (Cordell et al., 1998, 1999; Cornwell et al., 2007; Ekar et al., 2019; Morrison & Stacy, 2014; Sakishima, 2015). These ecological adaptations may be related to the variation in resistance to *C. lukuohia* infection observed among varieties. Hoof et al. (2008) found that xylem vessel diameters were similar between co-occurring trees of var. *glaberrima* and var. *polymorpha*, which could explain the similarities in mortality due to *C. lukuohia* infection. Vascular wilt pathogens generally colonize the xylem, so biochemical, anatomical and physiological defences that are specific to this tissue play a role in resistance to wilt diseases (Pouzoulet et al., 2014; Yadeta & Thomma, 2013). For instance, grapevine (*Vitis vinifera* L.) cultivars with narrower xylem vessel diameters were more effective at compartmentalizing *Phaeomoniella chlamydospora* (W. Gams, P. Crous, M.J. Wingf. and L. Mugnai) infection, resulting in smaller necrotic lesions than those observed on grapevine cultivars with wider xylem vessels (Pouzoulet et al., 2017). A comprehensive study of the xylem vessel diameters of the various *M. polymorpha* taxa has yet to be conducted, and further research including microscopy of cambial tissue from resistant and susceptible varieties may shed more light on the mechanisms behind the differences in disease susceptibility that were observed.

Discovering resistance to infection within the host population is an important step in mitigating the damage caused by fungal pathogens, especially for a dominant forest species such as *M. polymorpha*. Thus, the results from this study can be used as the basis for further, more comprehensive resistance screening projects. Once resistant individuals are identified, it might be possible to breed resistance into the different varieties of *M. polymorpha* that are more susceptible to ROD-induced mortality, allowing for outplanting of variety specific ROD-resistant plants in areas with harsh environments. Climate and environment are important considerations for selecting site-appropriate seedlings as noted in the planting of pines resistant to white pine blister rust (Schoettle & Sniezko, 2007). Since the four varieties of *M. polymorpha* on Hawai'i Island are adapted to contrasting environmental conditions, outplanting efforts will need to involve a range of environments and match variety to habitat carefully.

While the results of this study are promising, this work is just the first step towards understanding if resistance to *C. lukuohia* infection exists in *M. polymorpha* and if the varieties of this foundation tree respond differently to infection. The individuals of *M. polymorpha* used in this study were sampled from a few populations on east Hawai'i Island and therefore are not representative of the full genetic variation present within these varieties, three of which are broadly distributed across Hawai'i Island and some or all of the older islands. *M. polymorpha* var. *incana*, var. *glaberrima* and var. *polymorpha* also occur on the western side of Hawai'i Island, which is generally drier, and var. *glaberrima* and var. *polymorpha* occur in bogs on Kohala Volcano (Stacy et al., 2014). The effects of environment on disease-resistant traits are unknown, and thus, future resistance screenings should include material from these areas to encompass the breadth of *M. polymorpha* variability on Hawai'i Island. Future screenings should also include *M. waialealae* var. *fauriei* and var. *waialealae*, and *M. polymorpha* var. *dieteri*, var. *glaberrima* and var. *pumila* from Kaua'i since *C. lukuohia* was recently confirmed on that island (Brill et al., 2019.). Screening all *Metrosideros* taxa in the state would be the next logical step, leading into testing of *Metrosideros* across the Pacific. The results of this study suggest that resistance to *C. lukuohia* exists in *M. polymorpha* on Hawai'i Island. With further research and intervention we might be able to mitigate the effects of *C. lukuohia*-caused ROD on 'ōhi'a forests and protect the thousands of other native Hawaiian plants and animals that depend on this species for their survival.

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CONFLICT OF INTEREST

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PEER REVIEW

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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