

Rapid ‘Ōhi‘a Death in Hawai‘i

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1. Introduction

1.1 Natural history and importance of ‘ōhi‘a

Metrosideros polymorpha, Hawaiian common name ‘ōhi‘a, is the dominant tree of Hawaiian forests, comprising 80% of the native forests and 50% of all forests in the state (Owen et al., in press; US Department of Agriculture Forest Service, n.d.). ‘Ōhi‘a trees contain 41% of all live-tree carbon sequestered on these islands (Owen et al., in press; US Department of Agriculture Forest Service, n.d.). Many ‘ōhi‘a forests are almost monospecific in the canopy, although they support a diverse and largely endemic understory. ‘Ōhi‘a is one of the first woody species to colonize new lava flows and persists on highly weathered clays on the oldest islands (Mueller-Dombois et al., 2013). ‘Ōhi‘a forests dominate upper watersheds that can receive more than 10,000 mm annual precipitation and persist in dry forests receiving less than 800 mm annual precipitation. Their range is from sea level to more than 2400 m above sea level (Adee and Conrad, 1990). The largest ‘ōhi‘a trees can reach 30 m in height and 2 m in diameter (Fig. 15.1), while dwarf varieties growing in bogs can reach maturity at less than a meter in height. Hart (2010) calculated that the largest trees are over 600 years old.

‘Ōhi‘a forests provide an essential environmental service to the islands by protecting the upper watersheds. Water has long been recognized as the most important product of Hawai‘i’s forests (Hosmer, 1959). These forests also provide habitat for much of the endemic flora and fauna of Hawai‘i, including 21 species of endemic forest birds, 12 of which are endangered (Hawai‘i Department of Land and Natural Resources, 2015) (Fig. 15.2). In Hawaiian traditional plant lore, ‘ōhi‘a was a bodily form of the god Kū, and traditional statues of deities, or ki‘i, were carved from its wood (Abbott, 1992; Krauss, 1993; Kirch, 1985). Traditional and modern hula dancers often make flower garlands or leis of red, yellow, and orange ‘ōhi‘a flowers (Fig. 15.3). Posts and poles of ‘ōhi‘a are currently used in local architecture.

Currently, there are five recognized, endemic species of *Metrosideros* in Hawai‘i, with the most common species, *M. polymorpha*, having eight named varieties and one other species, *M. waialealae*, having two named varieties (Dawson and Stemmermann, 1999). An ancestral species of *Metrosideros* likely colonized the Hawaiian Islands from Tahiti or the Marquesas about 3.9 million years ago, and the species evolved into the diverse taxa known today, adapting to diverse habitats on different islands (Percy et al., 2008). Current genetic analysis has indicated that there is much more diversity in the Hawaiian *Metrosideros* taxa than is evident from the morphology and current taxonomy (Stacy and Sakishima, 2019).

Various large-scale mortality events have been documented in ‘ōhi‘a forests for over a century. Plant pathologist Harold Lyon reported widespread mortality of *Metrosideros* forests on Maui in 1909 (Lewton-Brain, 1909). Lyon attributed the decline to waterlogged soils and concluded that ‘ōhi‘a was not adapted to these soils and thus recommended replanting the forests with non-native species to protect the watersheds. In the 1950s through the 1980s, landscape-scale ‘ōhi‘a decline affected about 50,000 ha of forests on windward Hawai‘i Island (Hodges et al., 1986.) A team of pathologists, hydrologists, and entomologists concluded that dieback events began with an environmental stress, in most cases poor soil drainage but



FIG. 15.1 Large 'ōhi'a tree. (Photograph credit: J.B. Friday.)



FIG. 15.2 Crown and flowers of 'ōhi'a tree. (Photograph credit: J.B. Friday.)



FIG. 15.3 Hula dancer with lei (garlands) made of 'ōhi'a flowers. (Photograph credit to David Boyle/National Park Service.)

also including nutrient deficiencies, which predisposed trees to attack by other pests and pathogens. Both the native 'ōhi'a borer (*Plagithmysus bilineatus*, Cerambycidae) and the pathogenic fungi *Phytophthora cinnamomi* and *Armillaria mellea* were observed to attack and kill trees that were stressed, especially those growing in waterlogged soils (Papp et al., 1979; Papp and Samuelson, 1981; Kliejunas et al., 1977). No novel pathogens were found, however.

Mueller-Dombois (1983, 1985) developed a model of cohort senescence that assumed that the largely monospecific stands of 'ōhi'a that were dying represented single cohorts that regenerated after some disturbance. Upon reaching an age typical of the lifespan of the trees, these stands then succumbed to environmental stresses, pests, and pathogens. Lack of annual growth rings in the species makes it impossible to accurately age stands, but later observations of healthy regeneration in dieback stands (Boehmer et al., 2013) lend support to the cohort senescence model.

The recent discovery of the pathogen *Austropuccinia psidii* in Hawai'i (Uchida et al., 2006) represents a further threat to 'ōhi'a forests. While the fungus has decimated other plants in the Myrtaceae in Hawai'i, especially the non-native *Syzygium jambos*, until recently it had not been a particularly severe pathogen on 'ōhi'a (Loope, 2010; Loope and Uchida, 2012). In 2016, however, landscape-scale defoliation of 'ōhi'a in some locations on the islands of O'ahu and Moloka'i, seemingly by *Austropuccinia*, caused local foresters to re-evaluate the threat caused by the fungus (Weaver, 2021). It has also been found that *A. psidii* can interfere substantially with the growth of naturally regenerated and planted 'ōhi'a trees (Flint Hughes, US Department of Agriculture Forest Service, oral/written communication, 2014, and Aileen Yeh, Aileen's Nursery, oral/written communication, 2014) as young seedlings have younger, more vulnerable leaves, and as conditions near the ground can be very humid.

Many other fungi and bacteria have been found on 'ōhi'a (Hawai'i Ecosystems at Risk, 2005), but few of these have been implicated as being pathogenic. *Phomopsis* spp. and *Neofusicoccum* have been isolated in association with some cankers (Brian Bushe, University of Hawai'i at Mānoa, oral/written communication, 2020, and Marc Hughes, University of Hawai'i at Mānoa, oral/written communication, 2020) and can cause mortality of individual branches. Foliar pathogens found on 'ōhi'a that are associated with defoliation events include *Cylindrocladium*, *Phoma*, *Nigrospora*, and *Pestalotiopsis*.

2. A new disease of 'ōhi'a

Against this background of mortality from other causes, the first trees to die because of infection by *Ceratocystis* went largely unremarked. However, in 2012, foresters began receiving reports of unprecedented levels of 'ōhi'a mortality in

forests in the eastern district of Hawai‘i Island (Mortenson et al., 2016) (Fig. 15.4). High levels of mortality occurred in discrete areas interspersed with healthy forest. Because tree symptoms included rapid, synchronized death of leaves on individual branches that quickly spread to the entire canopy, the phenomenon was dubbed Rapid ‘Ōhi‘a Death (ROD) to contrast it from earlier dieback events that occurred more gradually (Fig. 15.5). Because the symptoms indicated water stress, roots were sampled for possible pathogens. Initially, both *Fusarium* and *Pythium* were recovered from roots of dying trees, but because both pathogens had long been common in the environment, they were not thought to be the cause of the recent mortality. The area where the mortality was first seen was on the slopes of Kīlauea, an active volcano, and a spatial analysis of mortality patterns in 2013 showed that all dieback patches were centered over fault lines (Friday, University of Hawai‘i at Mānoa, unpublished data). The hypothesis that the mortality was related to volcanic activity was discarded, however, as more outbreaks were found outside the volcanically active zones. In early 2014, wood samples from recently killed trees showing a dark staining of some xylem tissue were submitted to the University of Hawai‘i Agricultural Diagnostic Center in Hilo, where the pathogenic fungus *Ceratocystis* was isolated from the stained wood tissue (Keith et al., 2015) (Fig. 15.6).

Ceratocystis is certainly not a new genus of fungi in Hawai‘i. *Ceratocystis fimbriata* had been known for decades as a cause of black rot on sweet potato in Hawai‘i and as a minor pathogen on taro and the ornamental *Syngonium* (Uchida and Aragaki, 1979; Thorpe et al., 2005; Chung, 1923; Brown and Matsuura, 1941; Li et al., 2016, 2017). However, prior to these samples, *Ceratocystis* had never been isolated from ‘ōhi‘a. A first check to ensure that the *Ceratocystis* sp. being isolated

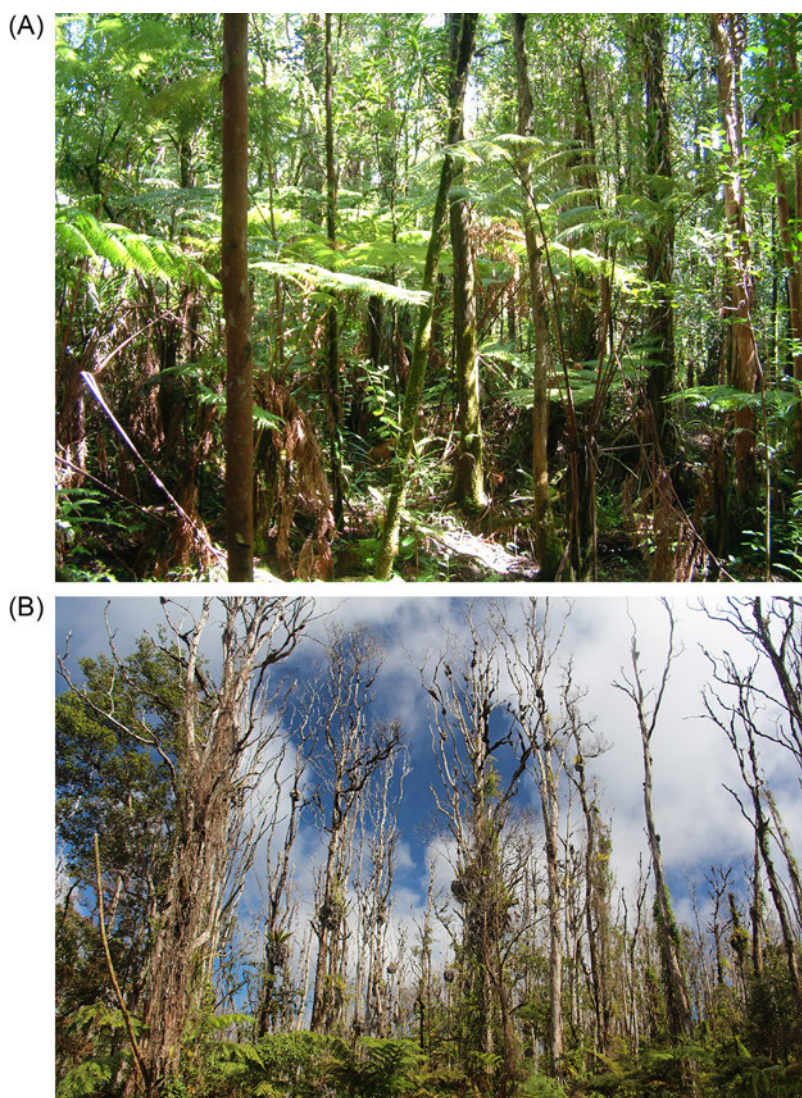


FIG. 15.4 (A) Healthy ‘ōhi‘a forest, Hawai‘i Island. (B) Same ‘ōhi‘a forest impacted by Rapid ‘Ōhi‘a Death 10 years later. (A and B: Photograph credit: J.B. Friday.)



FIG. 15.5 Tree showing Rapid 'Ōhi'a Death symptoms of brown leaves remaining in the crown compared with healthy trees. (Photograph credit: J.B. Friday.)



FIG. 15.6 Cross section of 'ōhi'a log showing typical staining. (Photograph credit: J.B. Friday.)

from dying 'ōhi'a was the primary pathogen causing the disease was to use a pure fungal culture of one of these isolates to artificially infect healthy 'ōhi'a and recreate the symptoms observed in the field. Researchers at the US Department of Agriculture, Agricultural Research Service pathology laboratory in Hilo, Hawai'i, followed Koch's postulates to confirm pathogenicity (Keith et al., 2015). Healthy 1- to 2-year-old 'ōhi'a seedlings were inoculated with a spore suspension in a stem flap wound, which was then wrapped with plastic. Wilt symptoms were observed in inoculated seedlings within 17–36 days and, as the disease progressed, leaves withered, died, and remained attached to the plant. After plant death, the seedlings were dissected, and internal discoloration was observed. These were the same symptoms observed in naturally infected 'ōhi'a trees. The same *Ceratocystis* was successfully re-isolated and identified from the infected seedlings, and all control plants remained healthy, confirming Koch's postulates. Based on a combination of disease symptoms typical of *Ceratocystis* infections, similar morphological structures to those of *C. fimbriata* sensu lato, and a 99% match to the internal transcribed spacer (ITS) sequences of *C. fimbriata* isolates in GenBank, the pathogen responsible for the wilt symptoms was identified as *C. fimbriata* and the disease was referred to as *Ceratocystis* wilt (Keith et al., 2015). Shortly after this work was completed, a second *Ceratocystis* isolate was obtained from dead and dying 'ōhi'a. Koch's postulates were completed as above, and the new *Ceratocystis* was also found capable of causing disease (Barnes et al., 2018).

Although no *Ceratocystis* problems were previously recorded for causing vascular wilt diseases in trees in Hawai'i, many *Ceratocystis*-caused vascular wilts occur worldwide. The genus *Ceratocystis* as currently defined is a closely related group of fungi that were once considered a single species, *C. fimbriata*. Today, the approximately 35 described species in the genus range from mild plant pathogens to aggressive tree killers (Barnes et al., 2018). There are at least four geographic

clades of *Ceratocystis*: the Latin American clade (LAC), the North American clade (NAC), an ill-defined African clade (AC), and the Asian-Australian clade (AAC) (Harrington, 2013). The most aggressive species of *Ceratocystis* are found in the LAC, which includes South American strains on mango and eucalyptus, which have been introduced to Asia (Oliveira et al., 2015). Also in the LAC is *C. platani*, which is native to the southeast United States but has been introduced to California and southern Europe, where it is an aggressive pathogen on *Platanus* species (Ocasio-Morales et al., 2007).

Researchers from the US Department of Agriculture, Agricultural Research Service pathology laboratory in Hilo, the University of Hawai‘i, and Iowa State University worked with scientists at the University of Pretoria using a combination of the phylogenetic, morphological, and biological species concepts, as well as pathogenicity tests and microsatellite analyses, to characterize isolates collected from diseased ‘ōhi‘a trees across Hawai‘i Island. From this work, two novel species of *Ceratocystis* were described: *Ceratocystis lukuohia* (destroyer of ‘ōhi‘a) and *C. huliiohia* (disruptor of ‘ōhi‘a) (Barnes et al., 2018). The Hawaiian-language specific names were given after consultation with Hawaiian cultural experts and marks the first time Hawaiian names were given to plant pathogens.

Further molecular genetic work found that *Ceratocystis lukuohia* is in the LAC group with the most aggressive pathogens and is very closely related to *C. platani*, along with the *C. fimbriata* strain attacking *Xanthosoma* found in the Caribbean, a strain attacking *Syngonium* that has been distributed widely (including Hawai‘i Island) in cuttings of this ornamental plant, and a Costa Rican strain on coffee (Barnes et al., 2018; Mayers et al., 2021). The extremely limited genetic variation found in *C. lukuohia* in Hawai‘i indicates recent introduction, perhaps on planting stock from Central America, the Caribbean, or southeastern United States (Harrington et al., 2021). The fungus could have jumped from another host, or mutated in a way that allowed it to infect *Metrosideros*, or developed as a hybrid between other strains/species already in Hawai‘i. In contrast, *C. huliiohia* is in the AAC and is closely related to *C. uchidae*, which has been found on taro, *Xanthosoma*, and *Eucalyptus* in China, Fiji, and Kaua‘i and O‘ahu (Li et al., 2017). *Ceratocystis uchidae* is not considered an aggressive pathogen, and *C. huliiohia* is much less aggressive on ‘ōhi‘a compared to *C. lukuohia* (Juzwik et al., 2019). *Ceratocystis huliiohia* appears to be widespread in the Hawaiian Islands and has likely been in Hawai‘i longer than *C. lukuohia* (Fortini et al., 2019). Usually, only one of the two *Ceratocystis* species is detected in a diseased tree, but in about 3% of the cases both fungi have been detected in the same tree (Heller and Keith, 2018).

3. Development of ROD symptoms on ‘ōhi‘a trees

Field experiments involving artificially inoculated trees with *C. lukuohia* or *C. huliiohia* and dissections of naturally infected trees (11–27 cm diameter at breast height) were initiated in Hawai‘i in 2017. For artificially inoculated trees, *Ceratocystis*-colonized filter paper or an agar media fungus slurry was placed on circular wounds made to the outer sapwood. The excised bark disk was then placed over the inoculum and sealed with epoxy resin (plumber’s putty). The results led to descriptions of the two diseases (Hughes et al., 2020; Juzwik et al., 2019). Within 2–3 months of artificial inoculation with *C. lukuohia*, trees (11–27 cm DBH) displayed a continuum of external symptoms ranging from asymptomatic to complete crown wilt with reddish-brown leaves attached (Hughes et al., 2020). Dissection of the trees revealed brown-to-black discoloration in the sapwood that appeared as longitudinal discontinuous spotting and streaking in the sapwood and extended radially inward through the rays at the leading edge of fungal colonization (Fig. 15.7A). In more extensively colonized tissue, staining formed a near-complete ring around the circumference and stain extended several centimeters inward in infected stems (Fig. 15.7C). As the tree became moribund, fungal colonization continued outward killing the vascular cambium and secondary phloem tissue, with the phloem changing from coral pink to brown in color. Pathogen movement through the vascular tissue, assessed by visible wood staining and fungal isolations, was rapid and routinely extended along the entire length of the tree. Dissections of naturally infected trees (25% and 75% crown wilt) yielded similar colonization patterns, with the fungus being found throughout the entire main stem (up to 13 m in height), and 60%–70% of the circumference of sampled stem disks showed characteristic staining. Movement of the pathogen was faster upward than downward and was likely due to passive movement of fungal propagules in the vascular system via the transpiration stream. Based on these findings, *C. lukuohia* was described as a systemic vascular wilt disease and named *Ceratocystis* wilt of ‘ōhi‘a (Hughes et al., 2020).

In contrast to trees inoculated with *C. lukuohia*, trees inoculated with *C. huliiohia* exhibited no crown symptoms typical of ROD within 54 or 90 days of inoculations occurring in late May or early August, respectively (Juzwik et al., 2019). However, upon de-barking and dissection of inoculated stem sections elliptically shaped, reddish-brown cankers of the inner phloem and cambium were observed with similar sized stained sapwood (Fig. 15.7B). The necrosis extended above and below the inoculated wounds, canker areas ranged from 243 to 450 cm² in size, and cankers (0.5 to 1.5 m long) were spiral in nature following the woodgrain. Naturally infected *C. huliiohia* trees exhibiting partial crown dieback were selected for dissection. Removal of the outer bark of stem sections and major limbs revealed multiple cankers on each tree. Coalescence of two or more cankers was observed on lower stems of two trees. Reddish-brown staining of



FIG. 15.7 Vascular staining patterns of artificially inoculated 'ōhi'a. Excised bolts and cross sections of trees infected with (A, C) longitudinal spotting and near-complete ring of staining in cross section of *C. lukuohia* colonized trees and (B, D) discrete elliptical (cankers) and wedge-like staining for *C. huliiohia* colonized trees. (Photograph credit: A, C: Marc Hughes; B, D: Jennifer Juzwik.)

the sapwood extended more deeply toward the stem center than dark staining associated with *C. lukuohia* infection. In cross section, the staining was amorphous, dark gray in color, and had a pie or wedge-shaped appearance (Fig. 15.7D). These observations indicate that multiple, repeated infections result in coalescing cankers, leading to crown dieback and tree death. Thus, crown wilt and tree death would occur more slowly than the systematic vascular wilt typical of *C. lukuohia*-infected trees. In contrast to *Bretziella fagacearum* (formerly *C. fagacearum*), the pathogen that causes oak wilt in the continental United States, neither *Ceratocystis lukuohia* nor *C. huliiohia* form mats of mycelia between the bark and wood (Juzwik et al., 2011).

The visual evidence of ROD-like symptoms, including a fading crown and the sudden browning and retention of the leaves, is not enough evidence to confirm *C. lukuohia* or *C. huliiohia* as the causal factor. 'Ōhi'a is known to be sensitive to physical injury that can manifest as ROD-like symptoms including crown decline and death (Friday and Herbert, 2006). Damage by heavy machinery can affect roots or the main stems and even light machinery like landscaping string trimmers can cause stem wounds that can girdle and kill 'ōhi'a. Exposed stem wounds can also serve as pathways for entry of stem and branch pathogens, and severe site stressors have been shown to trigger pathogenesis by endophytic latent pathogens that can produce brown-to-black vascular staining similar to ROD infection (Hughes et al., 2018).

Because ROD cannot be reliably diagnosed by visual symptoms alone, a quantitative/real-time (qPCR) diagnostic assay was developed to confirm the presence of each pathogen in samples. This assay targets the Cerato-platanin gene (Heller and Keith, 2018).

Trees are sampled by taking four drill bit samples of wood on the main stem of the suspected tree from the cardinal points at breast height (1.4m). The drill bit would be pushed to a depth of 5 cm into a tree, and the drill bit shavings would be carefully harvested in an Eppendorf vial and returned to the US Department of Agriculture, Agricultural Research Service laboratory where DNA is extracted for a quantitative polymerase chain reaction (qPCR) test that uses primers and probes to detect a portion of the Cerato-platanin gene (Heller and Keith, 2018). In addition, sawdust and/or wood samples are baited between two sterile carrot slices for 2 weeks (a technique developed by Moller and DeVay (1968a) to test for viability of fungus). All diagnostic samples submitted to the laboratory are screened by this qPCR test and also carrot-baited to test for viability. A different diagnostic system was subsequently developed to allow field crews to perform on-site diagnosis of *Ceratocystis* without having to take samples to the laboratory (Atkinson et al., 2017). This system, called a “lab in a suitcase,” uses isothermal DNA amplification with forward and reverse primers, recombinase polymerase amplification (RPA), and a fluorescent probe. The system can operate off of a 12-V battery and fits into the bed of a pickup truck. Diagnosis of *Ceratocystis* from wood shavings can be done in as little as 90 min in the field.

4. How the pathogen has been dispersed on Hawai‘i Island

Suspicious ‘ōhi‘a mortality was first noted in a subdivision on the eastern (windward) side of Hawai‘i Island in about 2010. Google Earth Imagery from January 21, 2013, depicts a substantial number of recently killed trees with brown leaves on the north and south sides of the main entrance to this subdivision, as well as many large, dead trees without fine branches, indicating that many trees had died before 2010. Aerial photographs from the same area show that there was much mortality from January 2010 through February 2012. The Google Earth 2013 imagery showed scattered dead trees throughout the area extending north into areas around the old sugarcane plantations west of Hilo town, on the east side of the island about 40 km away. After Tropical Storm Iselle hit eastern Hawai‘i Island in August 2014, there was increasing recognition of ‘ōhi‘a mortality. By 2016, ROD was detected from the middle of the eastern side of Hawai‘i Island to the southeastern corner of the island (Fig. 15.8), and by then, *C. lukuohia* was recognized as the cause. By 2016, one location on the west side of Hawai‘i Island also was showing signs of ROD.

Until January 2017, no substantial mortality had been noted north of Hilo town, but then two hot spots with substantial mortality were found on the windward side of the island, about 20 and 36 km north of Hilo. Isolates from these locations were genetically related to other isolates of *C. lukuohia* from the eastern corner of the island so it is hypothesized that the fungus was transported more than 50 km north by Tropical Storm Iselle. An even later episode, noted in late 2017, resulted in substantial new mortality 80 km northwest at the northern tip of the island, but this outbreak is not thought to be associated with Tropical Storm Iselle, and the isolates of *C. lukuohia* from this site are distinct from those from the eastern corner of the island and just north of Hilo (Harrington et al., 2021). Thus, the timing of the outbreaks and the genetic analyses of *C. lukuohia* isolates indicate that the disease may not be spreading slowly but instead taking major jumps.

New outbreak areas were noted in aerial surveys in 2018 and 2019 across Hawai‘i Island, and as of 2021, *C. lukuohia* was well established throughout Hawai‘i Island, although some of the more pristine ‘ōhi‘a forests at higher elevations on the northeast side of the island had not yet been substantially affected.

5. The spread to the other islands

Aerial surveys are conducted twice a year across the main Hawaiian Islands by the Hawai‘i Department of Land and Natural Resources to see whether ‘ōhi‘a on other islands have been affected by ROD. As of 2021, the following observations have been made and actions taken.

Maui: In 2019, one ‘ōhi‘a tree with *C. huliiohia* was found on the island of Maui, about 75 km northwest of the nearest disease site on Hawai‘i Island (Hawai‘i Department of Land and Natural Resources, 2019). The tree was planted in an agricultural area about 2 km from naturally occurring ‘ōhi‘a forests. The tree was felled, the stump excavated, and the wood burned. No further cases have been reported from that island.

O‘ahu: As of May 2021, there have been seven detections of *C. huliiohia* on the island of O‘ahu, all several kilometers apart (Hawai‘i Department of Land and Natural Resources, 2020b). Three have been in residential “backyard” plantings, and four have been in natural ‘ōhi‘a forests in the Ko‘olau Mountain range on the eastern side of the island. The first detection was made in July 2019. Managers have responded with felling, removal, and tarping when feasible. Monitoring of sites where disease detections have been made has not led to additional detections, indicating localized spread is low.

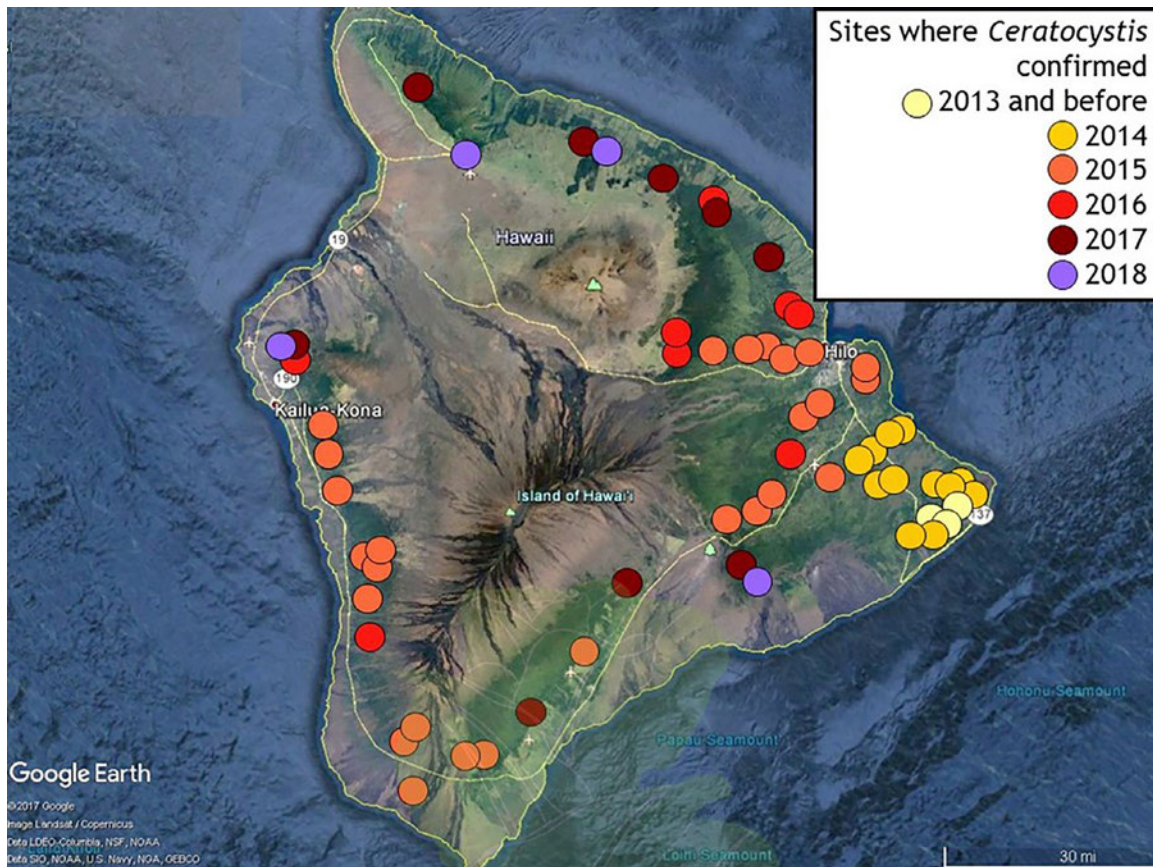


FIG. 15.8 Spread of Rapid 'Ōhi'a Death on Hawai'i Island. (Figure credit: J.B. Friday.)

Kaua'i: There are about 24 sites where 1–30 'ōhi'a trees have been found infected with either *C. lukuohia* or *C. huliiohia* (Hawai'i Department of Land and Natural Resources, 2020a). On Kaua'i, both fungi have been detected in dead trees about equally, although *C. huliiohia* is more widespread on the island and mostly *C. lukuohia* is detected in recent pockets of mortality. Some of the Kaua'i sites have been managed by felling and tarping diseased trees when feasible and continue to be monitored. The Kaua'i isolates of *C. lukuohia* are closely related to each other, indicating a single introduction, most likely from Hawai'i Island, whereas the *C. huliiohia* isolates do not form any geographic pattern, indicating that *C. huliiohia* has been on the island much longer (Brill et al., 2019; Heller et al., 2019; Harrington et al., 2021).

It was apparent by 2016 that many of the worst areas of ROD-caused mortality involved isolated and discrete patches of relatively large trees, most often trees with some crown exposure to the wind. In these patches, the trees appeared to be dying synchronously at first, but mortality continued for 2–3 years, after which the rate of new mortality generally tapered to a slower rate. Although *Ceratocystis* species have generally been thought of as insect-dispersed because of their ascospores that accumulate in sticky spore drops (Harrington, 2013; Moller and DeVay, 1968b), airborne transport of inoculum is the most likely explanation of these sudden patches of mortality, which were often many kilometers from any known source of inoculum. *Ceratocystis lukuohia* has been the predominant species isolated from such discrete mortality pockets, some of which have included hundreds of killed trees. *Ceratocystis huliiohia* does not appear to play a significant role in most episodes of mortality.

Several of the relatives of *C. lukuohia* in the LAC have been known to be aerially dispersed in the frass (feces and chewed fragments of wood) of ambrosia beetles, which often infest trees dying or killed by *Ceratocystis* (Iton, 1960; Harrington, 2013) (Fig. 15.9). In earlier work, *C. fimbriata* on mango and eucalyptus, *C. cacaofunesta* on cacao, and *C. platani* on oriental plane tree have been readily isolated from such ambrosia beetle frass after the trees have died (Harrington, 2013). It is hypothesized that dark, thick-walled aleurioconidia may survive in wood for years, and either these propagules or chlamydospores are believed to be the spore types dispersed in the ambrosia beetle frass, which is light and readily dispersed in the wind (M. Hughes., University of Hawai'i at Mānoa, written communication, 2020; Fig. 15.10).



FIG. 15.9 Frass produced by ambrosia beetles on an ‘ōhi‘a tree. (Photograph credit: Kylie Roy.)

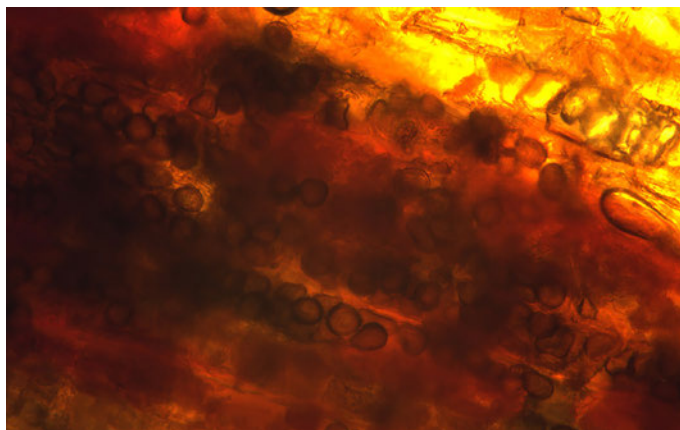


FIG. 15.10 *Ceratocystis lukuohia* aleurioconidia in *Metrosideros* wood. (Photograph credit: Blaine Luiz.)

Both *C. lukuohia* and *C. huliohia* have been isolated from ambrosia beetle frass, and Rotorod sampling and other air samples have detected the DNA of these fungi (Heller and Keith, 2018; Roy et al., 2019, 2020b). Soil samples from near ROD trees, from hooves of pigs, and scraped from boots far from ‘ōhi‘a mortality have also tested positive for these *Ceratocystis* spp., and ambrosia beetle frass is the likely source of this *Ceratocystis* DNA.

In general, trees infected and killed by *C. lukuohia* are dominant to codominant trees and have canopies with some exposure to wind. In many of the outbreak areas, there is a clear directionality to the crown exposure, such that the affected trees are all exposed to the same wind direction, which is typically not the direction of the prevailing winds. Further, when isolates from trees in an affected area are studied genetically, the isolates are closely related to each other, indicating that the trees were infected with inoculum from a single tree or small group of trees (Harrington et al., 2021).

Although wind-blown frass is hypothesized to be the dominant means whereby *Ceratocystis* spreads in Hawai‘i, other vectors may be involved in spreading the pathogens. While ‘ōhi‘a is seldom used for lumber, round logs are used for architectural posts and sold both on Hawai‘i Island and moved inter-island. Because the posts are not treated, transport of a post made from an infected tree could spread the pathogens. ‘ōhi‘a is increasingly used in landscape plantings, and nursery stock is also transported between islands. The Hawai‘i Department of Agriculture set up a quarantine on the movement of untreated ‘ōhi‘a logs, ‘ōhi‘a seedlings, and plant parts as well as soil to prevent these *Ceratocystis* fungi from moving from one island to another (Hawai‘i Department of Agriculture, 2016). As tree-infecting *Ceratocystis* spp. are thought to enter their hosts via stem and root wounds, several forms of mechanical transmission are likely involved in the infection process. The pathogens could be moved from tree to tree by arborist’s contaminated cutting tools, although planted ‘ōhi‘a trees have limited use in urban landscapes. The pathogens could be spread locally in rural, forested areas by machinery used by roadside vegetation

maintenance crews. Feral hooved animals (cattle, pigs, goats, and sheep) are common in Hawai'i forests, and the wounds they produce during rooting and rubbing could serve as important infection courts. The role of these animals in direct fungal spread and transmission is under investigation. *Ceratocystis platani*, which affects plane trees (*Platanus* spp.) in Europe, moves down waterways (Harrington, 2013; Tsopelas et al., 2017), but transport down the short, usually intermittent streams on Hawai'i Island does not seem to be a main mode of spread, although waterway movement on older islands with more perennial streams has yet to be investigated. The pathogen is not thought to spread through root contact with neighboring trees, as *C. platani* and *Bretziella fagacearum* are spread (Panconesi, 1999; Juzwik et al., 2011; Harrington, 2013), because of observations of how the diseases spread intermittently through a stand, rather than infecting one tree and then going on to the next. The pathogen has been detected in soil as have many other *Ceratocystis* pathogens (Harrington, 2013; Heller and Keith, 2018; Yelenik et al., 2020). There is mounting evidence that some hooved feral animals (wild pigs, goats, cattle) may be involved in the spread of the pathogen and the wounding of 'ōhi'a trees, which makes them more vulnerable to infection (Perroy et al., 2021).

Similar to other *Ceratocystis* diseases, wood-boring beetles are suspected of being involved with the spread of ROD. On Hawai'i Island, eight species of ambrosia beetles (Coleoptera: Curculionidae) have been reared from ROD-infected bolts (tree stem sections) and the five most common are *Xyleborinus saxesenii*, *Xyleborus affinis*, *Xyleborus ferrugineus*, *Xyleborus perforans*, and *Xyleborus simillimus* (Roy et al., 2020b) (Fig. 15.11). All five of these beetle species are in the tribe Xyleborini, a group associated with invasion due to their parthenogenic reproductive behavior (Kirkendall, 1993). A monophyletic radiation of native Xyleborini has established in Hawai'i, and *X. simillimus* is the only described native on 'ōhi'a (Samuelson, 1981). This species is limited to high elevations above 700m, a range that has shrunk considerably in the last 40 years (Roy et al., 2020b).

These five ambrosia beetle species may be involved with the spread of ROD in two ways: indirect transmission and direct transmission. Indirect transmission refers to the potential movement of *Ceratocystis* propagules in free frass particles (wood-boring dust), and direct transmission refers to the movement of fungal spores by ambrosia beetles, presumably carried on their exoskeletons. The movement of contaminated frass and detection of *Ceratocystis* on the exoskeleton of beetles have been documented in the *C. cacaofunesta* (Iton, 1966) and *C. platani* (Ocasio-Morales et al., 2007; Tsopelas et al., 2017) pathosystems. However, the importance of direct transmission into the host by burrowing ambrosia beetles has generally not been considered as important as frass dispersal by wind and rain, which appears to be a major dispersal mechanism for several members of the LAC (Harrington, 2013).

During gallery excavation, ambrosia beetles bore into the xylem of 'ōhi'a trees and push out viable *Ceratocystis* fungal propagules held within the frass into the environment (Roy et al., 2019). These frass particles can be transported in the wind, soil, and water and potentially infect other trees. All five of the common ambrosia beetle species found attacking ROD-infected trees produce frass that can contain viable fungal propagules (Roy et al., 2020a). In growth chamber experiments, *C. lukuohia*-infested ambrosia beetle frass was able to induce wilt in wound-inoculated seedlings although disease transmission was highly variable (M. Hughes, University of Hawai'i at Mānoa, written communication, 2021). Temperature and age play a significant role in the survival of *Ceratocystis* propagules embedded in frass, and propagules can remain viable for over 6 months under cool and high humidity conditions (M. Hughes, University of Hawai'i at Mānoa, written communication, 2021).



FIG. 15.11 *Xyleborus simillimus*, a native ambrosia beetle associated with the spread of Rapid 'Ōhi'a Death. (Photograph credit: Robert Peck.)

Ceratocystis lukuohia and *C. huliohia* DNA was detected and ambrosia beetle frass particles found in air samplers, supporting the idea of short-range airborne dispersal of the fungi in frass (Atkinson et al., 2019; Roy et al., 2021). Factors such as frass particle size and shape, wind speed, rain events, and the height at which beetles colonize trees likely affect long- and short-distance wind dispersal patterns of *Ceratocystis* propagules embedded in frass. *Xyloborinus saxesenii* produces 3.8 ± 0.8 mg of frass per day and may be the most likely to produce frass for long-distance spread as it can occupy the whole length of the bole of infected trees (Roy et al., 2020b). *Xyloborus ferrugineus* produces 7.8 ± 0.8 mg of frass each day but only occupies the base of trees and may be more likely to contribute only to short distance spread (Roy et al., 2020b). It is still not clear how far inoculum can be spread by wind.

Although *Ceratocystis*-infected frass moving in the wind is hypothesized to be the main way these pathogens are transported to new areas, presence of inoculum is only one of the conditions for new infections to get started. *Ceratocystis* species are thought to be dependent on wounds for ingress and infection of tree stems (Harrington, 2013), and that is a likely requirement for these fungi to infect ‘ōhi‘a. Observations through dissection of trees indicate that roots are not commonly infected by either *Ceratocystis* species. *C. lukuohia* and *C. huliohia* both infect stem wounds in inoculation studies, and *C. lukuohia* has been isolated from branches in the canopy of naturally infected trees. Many infections are likely through branch wounds or broken branch tips, but larger branches also may be infected. Heavy colonization (heavy sapwood staining) by *C. lukuohia* of branch junctions with included bark has been commonly observed in dissections of dead and dying trees. Young ‘ōhi‘a trees typically have multiple upright branches that fuse to form single stem years later. This area between the upright branches where the old bark is included is a weak spot on the stem, and such branch crotches at these junctions would be expected to crack open as the tree twists in wind storms. Dissection of killed trees, particularly the first trees killed at a site, has often shown the most extensive and oldest staining induced by *C. lukuohia* at such branch junctures (Harrington, Iowa State University, personal observation, 2016). It is believed that such infections lead to relatively quick death, while infections of smaller branches may not kill trees for years because the fungus moves quickly upward in trees but only very slowly downward (Juzwik et al., 2019; Hughes et al., 2020).

Wind is a frequent source of tree damage in Hawai‘i. Tropical storms, in particular, can cause stand-level damage with the most recent example being Tropical Storm Iselle that caused widespread tree damage on Hawai‘i Island in 2014. The area damaged by the storm has experienced some of the highest levels of ROD-caused mortality on the island. Wind can cause injuries that are directly infected by wind-blown frass, but injuries also can attract ambrosia beetles carrying inoculum.

According to the disease triangle concept, a disease involves vulnerable host tissue, environmental conditions conducive to infection, and the presence of the pathogen. Because of dominant and at times unexpectedly variable wind currents, many of the trees on Hawai‘i Island might be able to receive some inoculum in the form of frass, although the amounts of inoculum could vary substantially at different locations. While ‘ōhi‘a forests cover much of the landscape of Hawai‘i, particularly in upland areas, there are variations in the numbers and sizes of trees and variations in the amount of wounded tissue they could present to arriving inoculum. To date, no surveys are available that could be used to document the amount of wounded tissue that might be present in any given stand of ‘ōhi‘a; however, several reasons and evidence indicate that differences in these parameters could be sizable. For example, these diseases became more apparent after Tropical Storm Iselle landed on the windward side of the island in 2014 and caused limb breakages in hundreds of thousands of trees. The next 4 years after tropical storm Iselle were when most of the ‘ōhi‘a that died from ROD succumbed. In the past 3 years, during which few violent winds occurred, rates of mortality have stabilized even though a great number of dead ‘ōhi‘a trees are infested with ambrosia beetles that are pushing out frass. This indicates there have been relatively fewer storm-damaged ‘ōhi‘a trees to infect.

Of course, when a tropical storm occurs, the damage that it causes can also be highly variable. The focal points for the random outbreak events could be places where the storm happened to touch down. Large emergent trees, trees on the outside of a stand, and trees with steep-angled bifurcated stems would all be subject to much greater levels of damage if hit with a strong wind and therefore would be more vulnerable to colonization by *Ceratocystis* than smaller trees with compact crowns and plenty of neighboring trees and no bifurcations. Field plot data has shown that trees of diameter of <20 cm are less likely to be infected with *Ceratocystis* than larger trees (Hughes, 2021).

6. Monitoring of the spread of the disease

While all these etiological studies were taking place, the effect the diseases were having across the remainder of Hawai‘i Island and the rest of the state was also being monitored. Information from this work is not only useful for showing how the diseases were spreading, but also to help inform where implementing control measures might be most effective.

Soon after ROD was recognized, efforts were initiated to raise awareness of the new diseases among natural resource professionals, foresters, and land managers. Word spread quickly through professional meetings and networks. Within a year of

the discovery of the pathogens most foresters, ecologists, arborists, and professional land managers were aware of ROD and were able to recognize the symptoms (Friday, 2015). Private landowners, and native Hawaiians in particular, were deeply concerned about the loss of 'ōhi'a forest, especially as 'ōhi'a has traditional cultural connections (Kealiikanakaolehailani et al., 2018). Reaching private forest landowners, many who live in remote locations, was accomplished through hundreds of community meetings across the island (Friday et al., 2015), informational booths at local fairs and events, radio announcements, a website (<https://cms.ctahr.hawaii.edu/rod/>), social media, and production of a half-hour documentary film called "Saving 'Ōhi'a: Hawaii's Sacred Tree". The informational outreach efforts were effective: After 3 years, a statewide poll showed that 92% of the residents of Hawai'i Island knew about ROD and 48% of all residents of the state, including urban Honolulu, had heard of the diseases (Coordinating Group on Alien Pest Species, 2017). Reports from both professionals and community forest users subsequently led to new detections of the diseases not only on Hawai'i Island but also on Maui, O'ahu, and Kaua'i (Hawai'i Department of Land and Natural Resources, 2019, 2020a,b). Initial reports of the diseases were based on roadside surveys. The visible symptom of the crowns of entire trees turning brown over the course of a few weeks was unique and easily observed. However, only a few forest trees can be seen from a roadside.

The next step was to estimate landscape-scale spread of the diseases by using available aerial photographs (Mortenson et al., 2016). Subsequently, Asner et al. (2018) were able to develop spectral signatures that enabled them to map areas of 'ōhi'a mortality on an island-wide basis, covering >500,000 ha, using sensors on fixed-wing aircraft (Vaughn et al., 2018). These surveys picked up mortality caused by other factors such as drought, in addition to ROD, and thus did not provide an estimate of the extent of ROD. Further analysis of imagery combined with field sampling has shown that most but not all stands exhibiting spectral symptoms of ROD are indeed infected with *Ceratocystis* (Perroy et al., 2021).

These large-scale mapping efforts have been invaluable in illustrating the broader spatial extent and patterns of 'ōhi'a mortality across Hawai'i Island, although the infrequency of flights limits their utility for repeat monitoring to detect new outbreaks. Aerial surveys are flown twice a year across all 'ōhi'a forests in the state using digital mobile sketch mapping (DMSM) (Potter and Conkling, 2017) to identify new outbreaks on the basis of visible crown symptoms. These helicopter-based observations provide rapid assessments of forest health over large (>100,000 acre) areas, but their semiquantitative nature does not result in spatially explicit data. Detections of visually symptomatic trees in new areas are followed up by field sampling to confirm infection by *Ceratocystis*.

Small unmanned aerial systems (sUAS), carrying both visible and multispectral sensors (Perroy et al., 2020, 2021), can produce very high spatial resolution imagery (<2 cm) and have been used to detect new outbreaks and monitor disease progression (Fig. 15.12) and spread. sUAS have also been used by ground crews in real time to accurately and safely navigate to trees identified as targets for sampling from previous aerial surveys. sUAS platforms produce valuable imagery, but their

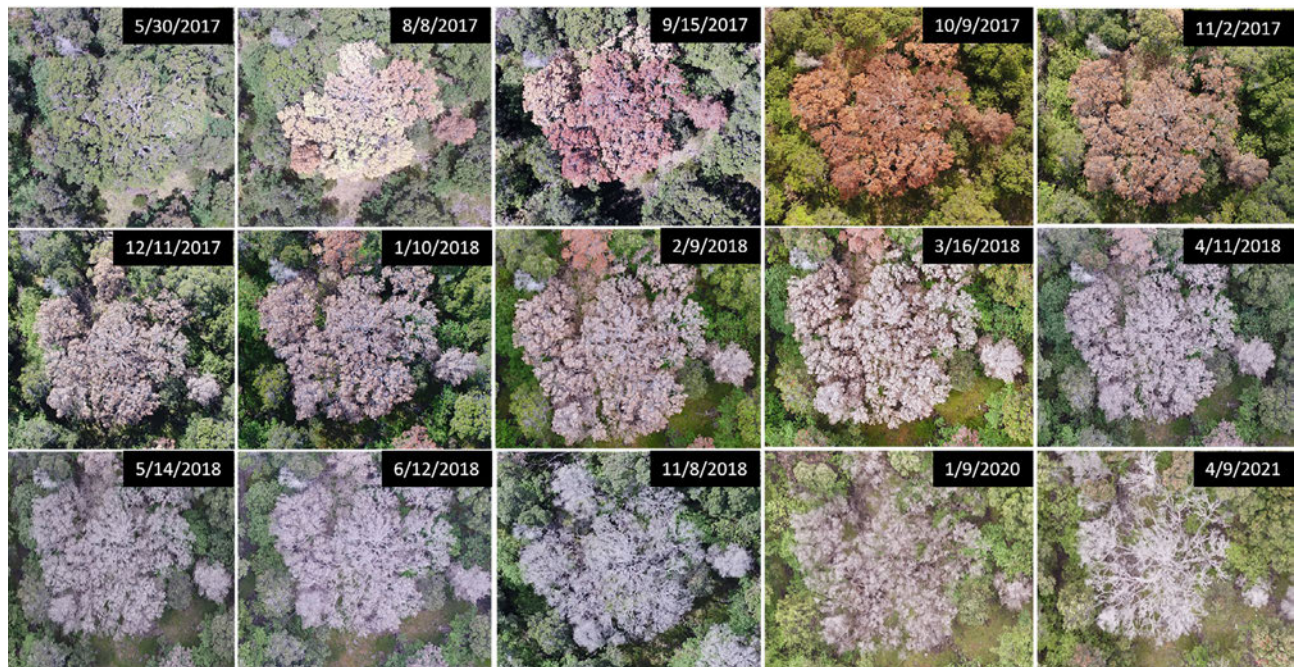


FIG. 15.12 Progression of canopy symptoms for a tree infected with *Ceratocystis lukuohia*. Aerial imagery collected from a long-term monitoring plot in the Ka'u District of Hawai'i Island. (Photograph credit: Ryan Perroy.)

area of coverage is limited by flight times and current regulations relating to visual line of sight (Hardin et al., 2019). A helicopter-based visible and near-infrared wavelength imaging system, developed by the University of Hawai'i at Hilo and the U.S. National Park Service, has been used to collect <5 cm imagery over large areas (10,000s of ha) on the islands of Kaua'i, O'ahu, and Hawai'i. This system has been used for monitoring and outbreak detection in areas too remote or expansive to be reasonably surveyed via sUAS platforms. Multispectral satellite imagery with <50 cm spatial resolution shows great promise for monitoring large areas across the Hawaiian Islands, although reliable access to imagery and regions with persistent cloud cover presents issues for widespread adoption for management. A remote sensing approach that combines sUAS, traditional fixed-wing and helicopter, satellite-borne sensors, and artificial intelligence detection algorithms to quickly process these data would help achieve a more robust and comprehensive monitoring program. Despite the technological advances, final diagnosis of the cause of these ROD-like symptoms still requires a PCR test on a sample of wood.

7. Effects of ROD on 'ōhi'a forests

Rapid 'Ōhi'a Death has been detected across most of the environmental envelope, in terms of temperature and rainfall, of the 'ōhi'a forest (Fortini et al., 2019). Only the coolest and the driest areas remain free of ROD. Projections indicate that most 'ōhi'a forests across the state will be highly susceptible to the disease.

Researchers with the US Department of Agriculture Forest Service Institute of Pacific Islands Forestry have established a network of 250 0.1-ha inventory plots in diverse 'ōhi'a forests across Hawai'i Island in order to monitor disease effects, patterns, and distribution (Hughes, 2021). Plots are located in areas where *Ceratocystis*-infected trees have been detected or are suspected to occur. Sites encompass a wide range of soil substrate ages (volcanic ash or lava from 64 to 190,000 years before present), rainfall (from 798 to 6273 mm), and mean annual temperatures (from 13 to 23°C). Plots are inventoried annually to record change in tree conditions and characteristics (for example, trunk diameter) of both living and dead trees.

In forest stands where *C. lukuohia*-infected trees occurred, 'ōhi'a annual mortality rates averaged 9% based on stem count and 10% based on loss of basal area with stem mortality (Hughes, 2021). In contrast, annual 'ōhi'a mortality rates were only about 2% in forest stands where only *C. huliiohia*-infected trees were present and about 2% where neither *Ceratocystis* species was detected but where trees showed ROD-like symptoms. Individual plot annual mortality rates ranged from 44% to none at all in some plots, even though *Ceratocystis*-infected trees were confirmed present.

Overall levels of 'ōhi'a mortality on a given plot where *C. lukuohia*-infected trees occurred were strongly related to the annual rate of mortality on that plot. That is, higher rates of mortality are found in areas where high levels of mortality have occurred, indicating that intrinsic site characteristics might be conducive to the diseases. Results also clearly showed that smaller stature 'ōhi'a stands, i.e., stands where average stem diameter at breast height <20 cm, exhibited lower annual rates of mortality of 4%–5% compared to larger-statured 'ōhi'a stands where mortality rates of 12%–13% were typical (Hughes, 2021).

Mean annual mortality rates of plots based on stem mortality were negatively related to increasing elevation and positively related to increasing mean annual temperature. Mean annual mortality rates were also positively related to mean annual precipitation (MAP), where mortality rates steadily increased from relatively low values in drier areas to higher values in stands experiencing MAP values in the 2500–3500 mm range. 'Ōhi'a stand level mortality rates tended to be lower in drier and cooler areas relative to their warmer and wetter counterparts (Hughes, 2021).

8. Economic impacts of ROD

While no formal economic analysis of the costs and effects of Rapid 'Ōhi'a Death has been completed to date, the loss of ecosystem services is significant and potentially catastrophic. Chief among these costs would be decreases in watershed recharge. Rainfall in the upper, forested watersheds is the only source of fresh water in the Hawaiian Islands. Communities in the leeward sides of Hawai'i Island are already pumping water at close to the replacement rate; any loss of recharge could result in overdrawing and having seawater intrude into some wells (Brauman et al., 2015; Engott, 2011). Kaiser et al. (1999) calculated that if water on O'ahu had to be supplied by desalinization rather than rainfall recharging the aquifers, costs would be between \$4 billion and \$8 billion dollars net present value. The faster-growing non-native species that would take over forests if 'ōhi'a forests were lost to ROD would have higher rates of evapotranspiration and would likely decrease watershed recharge (Takahashi et al., 2011). The negative visual effects of dead trees surrounding houses are causing losses in property values for forest landowners. Tens of thousands of Hawai'i Island homeowners will potentially be affected. Widespread forest mortality could also have a negative effect on tourism. Over a million visitors a year visit Hawai'i Volcanoes National Park (2019), certainly to see the geology but also to experience the rainforest with its unique flora and fauna (Hawai'i Volcanoes National Park, 2022). If a substantial number of tourists were discouraged by seeing forests devastated by ROD, the economic effects on local tourism dependent communities could be significant. Lastly,

while it is difficult to quantify the economic cost of threats to endangered species, the costs would be significant (Fortini et al., 2019). If enough habitats were lost, management agencies could be forced to expand expensive captive breeding programs for endangered forest birds and propagation facilities for endangered plants at the cost of millions of dollars (US Fish and Wildlife Service, 2009).

9. Managing the spread of the pathogens

Control methods have been developed that could slow the spread of ROD and potentially save much of the 'ōhi'a forest. Nothing can slow wind speeds or alter the path of tropical storms that occasionally hit some parts of Hawai'i, and indeed, climate change experts and models indicate an increase in storm activity and intensity (Murakami et al., 2012). However, other actions can be done to reduce the spread of the pathogens.

One of these is to reduce the spread of infective ambrosia beetles and ambrosia beetle frass from diseased trees. Forest managers with the state Division of Forestry and Wildlife and with Hawai'i Volcanoes National Park have felled hundreds of infected trees to reduce the dispersal of wind-blown frass. While beetles continue to be active in felled trees, the amount of frass picked up by the wind stream is reduced when trees are on the ground. Initially, efforts were made to fell trees at the leading edge of an outbreak in order to limit the spread of the disease into adjacent forest areas, but new evidence has shown that the diseases do not move gradually across the landscape but rather jump tens of kilometers at a time, likely driven by storms (Harrington et al., 2021). Felled trees can also be tarped to further reduce spread of frass, but in areas where dozens of trees are being felled tarping would greatly add to the labor cost. On the other islands with only a few instances of ROD, infected trees are felled and tarped or even excavated entirely and burnt (Hawai'i Department of Land and Natural Resources, 2019, 2020b). Felling of trees in dense stands is also avoided after experiences where healthy trees damaged by felled trees subsequently became infected. Over time, managers have developed a decision tree to guide workers on how to manage infected trees (Fig. 15.13).

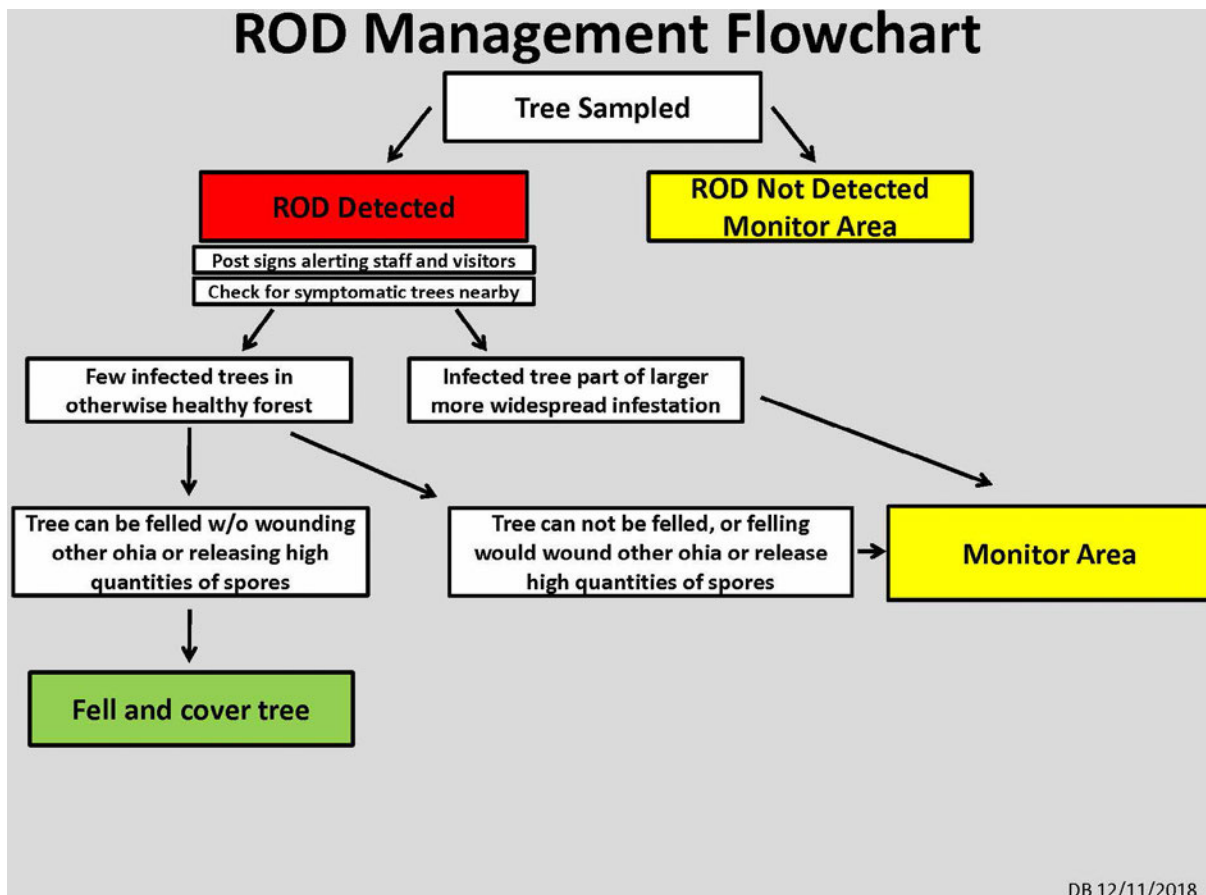


FIG. 15.13 Rapid 'Ōhi'a Death management flow chart. (Photograph credit: David Benitez.)

A few other measures have also been tried to slow the spread of ROD on an experimental scale. The use of broad-spectrum insecticides to prevent beetle attacks on both standing trees and felled logs has been tested, although the longevity of the application in the humid, wet conditions of Hawaiian rainforests is questionable. Roy et al. (2021) found bifenthrin to be effective at reducing beetle attacks for 3 weeks, and the further use of insecticides as a preventive measure is currently under investigation. Currently, no formulations of bifenthrin are registered for forestry use in Hawai‘i.

The use of semiochemicals (chemical compounds used in biological communication) is an environmentally friendly and pest-specific alternative to the use of broad-spectrum insecticides. Semiochemicals are widely used for the management of insect pests, particularly in agriculture crops and to a lesser extent in forest pest management. Integrated pest management tools that incorporate the use of semiochemicals include those used for mating disruption as attractants (aggregation pheromones) to traps for eradication or monitoring and as repellents (anti-aggregation pheromones) to deter insect attacks or feeding. The commercially available anti-aggregation semiochemical verbenone is an effective repellent of many ambrosia beetle species, including *Xyleborus ferrugineus*, *X. affinis*, and *Xyloborinus saxeseni* (Rivera et al., 2020). The use of verbenone and other semiochemicals is currently being investigated for potential use in *Ceratocystis*-infected ‘ōhi‘a.

Trade in ‘ōhi‘a wood products could be another pathway for movement of the pathogens, on either the island or the inter-island. Posts and poles for architectural use are routinely shipped from Hawai‘i Island to Maui, O‘ahu, and Kaua‘i. *Ceratocystis* has been shown to be viable in felled trees for several years (Harrington, 2013; Peck et al., 2021), so movement of posts poses a particular concern. In 2015, the Hawai‘i Department of Agriculture (2016) imposed a quarantine on Hawai‘i Island prohibiting the export of live plants and any untreated product made from ‘ōhi‘a. Kiln-drying sufficed to kill fungal spores in wood, but as posts are generally untreated, the Hawai‘i Department of Agriculture has mandated that businesses wishing to ship posts inter-island need to have them individually tested for DNA evidence of *Ceratocystis*. Posts testing positive may not be shipped off-island, although they may be used on island.

A set of experiments was conducted to determine the most cost-effective treatment of ‘ōhi‘a logs to prevent inter-island transport of inoculum. Borate and quaternary ammonia dips were tried first, but these did not penetrate the wood well and were not sufficient to completely kill the *Ceratocystis* deep in the log (Hughes et al., 2021). A vacuum steam process using a 56°C/30-min schedule was effective in eradicating *Ceratocystis* spp. in >99.5% of sapwood samples assayed. A 60°C/60-min schedule resulted in total eradication of *Ceratocystis* in sapwood of ‘ōhi‘a logs colonized by the ROD pathogens (Juzwik et al., 2021). In these tests, 22- to 44-cm-diameter (small end) logs were obtained from naturally infected ROD trees and subjected to the indicated heat treatment. Wood samples were then taken to depths of 70% of the log radius for evaluation. Additional studies using a dehumidification kiln for smaller diameter logs were successful at eradicating both ROD fungi when using a 56°C/30-min and 60°C/60-min schedule, respectively (M.A. Hughes, University of Hawai‘i at Mānoa, unpublished data).

Forest managers have also been concerned that the pathogens could be spread by movement of frass-contaminated soil on vehicles, footwear, or even feral animals. Local land management agencies, scientists, and other professionals rapidly adopted stringent bio-sanitary protocols including pressure-washing vehicles after travelling off-road in infected forests and cleaning boots and cutting tools with 70% isopropyl or ethanol (Roy et al., 2020a). Websites (<https://cms.ctahr.hawaii.edu/rod/>) and videos (<https://www.youtube.com/watch?v=CU6GH4PH-7I>) have been published to encourage good bio-sanitation. These efforts are not only aimed at stopping the spread of *Ceratocystis* but also of insect pests and weed seeds and have gained widespread support. Targeted efforts have also been made by the University of Hawai‘i Cooperative Extension Service and island-based Invasive Species Committees to reach eco-tour companies who bring visitors to remote and often pristine areas. A series of workshops was held statewide that educated over 100 tour operators on how to avoid spreading weeds, pests, and diseases. Tour operators in turn are able to reach hundreds or thousands of guests on an ongoing basis. Agencies such as the Hawai‘i Department of Land and Natural Resources and US National Parks have also been installing boot-brush stations at major trailheads statewide, starting on Hawai‘i Island and including state trails, parks, and the National Parks (see, for example, <https://www.nps.gov/havo/learn/nature/rapid-ohia-death.htm>). These stations are meant to reduce the spread of weeds and pests into the forests as well as reducing the spread of soilborne pathogens. The Hawai‘i program borrows heavily from the kauri dieback program in New Zealand both in the techniques used and in community messaging (see <https://www.kauriprotection.co.nz/>).

10. Management of ‘ōhi‘a forests

The potential for systemic fungicides and delivery systems used to manage laurel wilt in redbay (*Persea borbonia*) (Mayfield et al., 2008) and oak wilt in *Quercus* species (Blaedow, 2010) to manage Rapid ‘Ōhi‘a Death was investigated for their ability to prevent ROD symptom development in ‘ōhi‘a. Field experiments were conducted at two sites, 22 and 1650 m elevation on the windward side of Hawai‘i Island between May 2018 and February 2021. Macro- and/or micro-infusion methods

were used to inject propiconazole (14.3% a.i.) (PPZL) into healthy 'ōhi'a 1 month prior to challenge inoculation with *C. lukuohia* (Keith, 2020). Results varied at different sites with the application at the high-elevation site being more successful than at the low-elevation site. Environmental conditions and 'ōhi'a variety both play an important role in effectiveness of living tree treatments and indicate that more than one application is necessary to prevent ROD symptom development. While this would not be considered a viable approach for management of the disease in a forest, systemic fungicides could be used to protect high value 'ōhi'a in residential, urban, or park settings.

Because wounding seems to be necessary for trees to become infected, efforts are being made to protect forests from wounding at a landscape scale. Wind has been focused on as a main source of wounding in 'ōhi'a trees and is certainly very important, but it cannot be controlled. However, there are many other causes of wounding from anthropogenic causes that can be controlled. Clearing roads using heavy machinery, careless machete use, and string trimmers are among the most common causes of the human-inflicted wounds on 'ōhi'a. Statewide extension programs have been focused on reducing the unintentional wounding of 'ōhi'a trees, especially within the arboriculture community.

Wounds from feral and domestic animals can cause substantial damage to trees. Pigs, cattle, sheep, and goats were introduced to Hawai'i during the late 18th century and have since spread throughout the islands. Large herds of feral goats inhabit most dry forests, and extensive pig rooting is evident throughout most wet forests. A recent study has shown that these animals may have some role in wounding 'ōhi'a and/or spreading ambrosia beetle frass and making Hawai'i's 'ōhi'a forests more vulnerable to infection by *Ceratocystis* spp. (Perroy et al., 2021).

Indeed, in regions with confirmed cases of *C. lukuohia*, striking differences in 'ōhi'a mortality have been observed across fence lines separating adjacent areas with and without ungulates (Perroy et al., 2021) (Fig. 15.14). Based on high-resolution remotely sensed data, 'ōhi'a mortality levels have been observed to be up to 69 times greater in areas with ungulates compared to neighboring ungulate free areas (Perroy et al., 2021). Dramatic increases in 'ōhi'a mortality levels have also been observed within fenced areas that experienced subsequent fence breaches and animal incursions, highlighting the importance of fence line monitoring and maintenance. Because no game animals are native in Hawai'i, feral hooved animals provide opportunities for both recreation and subsistence hunting for local communities. The fencing and removal of ungulates have caused conflict with the hunting community in the past, and ongoing discussions with hunting groups may be helpful to reach consensus as to which forests could be managed for hunting and which for protection of the native biota.



FIG. 15.14 sUAS image along a fence line showing extensive 'ōhi'a mortality on the left side (ungulates present) and little mortality on the ungulate-excluded right side. Exposed fence can be seen in the lower portion of the image. Photograph collected on April 9, 2021. (Photograph credit: Ryan Perroy.)

Efforts are underway by the Hawai'i Department of Land and Natural Resources to protect forests with high native biodiversity while increasing hunter access to other forests with less biodiversity but with opportunities for game management.

11. Regeneration of 'ōhi'a following ROD

The natural regeneration of 'ōhi'a forests following ROD devastation is as of yet uncertain. The sites with heaviest ROD mortality tend to be in wet forests at low elevation (Fortini et al., 2019) with a history of disturbance (Mortenson et al., 2016). These factors covary with a high density and diversity of invasive plant species in the understory and overstory (Mortenson et al., 2016). In a recent survey of plots affected by stand-level 'ōhi'a dieback in the 1970s, Mertelmeyer et al. (2019) found that 21% of resampled forest plots did not return to an 'ōhi'a-dominated condition. This was likely due to invaders such as strawberry guava (*Psidium cattleianum*) in the understory, which have faster growth rates than 'ōhi'a and can overtop native seedlings quickly and ultimately lead to type conversion (Mueller-Dombois et al., 2013). Longer-term monitoring of natural recruitment looking to pre-invasion status as a factor may help elucidate the importance of considering pre-ROD invasions when determining post-ROD management. The ongoing study with 250 long-term plots in the ROD-affected 'ōhi'a forests found that 'ōhi'a seedlings were absent in 80% of all forest plots, including all plots below 1000 m (Hughes, 2021).

In forests dominated by invasive species, where little natural 'ōhi'a recruitment is expected, one management tool would be to plant 'ōhi'a. Previous research indicates that successful restoration of highly degraded forests would involve intensive management due to the nature of the presence of aggressive weedy plant species and non-native ungulates in these forests (Cordell et al., 2009; Ostertag et al., 2009). Planted 'ōhi'a can be quite vulnerable to the *Austropuccinia psidii* rust (Killgore and Heu, 2007). Recent work by Yelenik et al. (2020) compared mortality due to ROD, competition from invasive plant species, and disturbance by feral ungulates by planting 'ōhi'a seedlings into a forest site severely affected by ROD. The study experimentally manipulated competition and disturbance via weeding and/or fencing treatments around a subset of the 'ōhi'a seedlings. Seedlings were monitored monthly, and ROD presence was determined via laboratory methods if a seedling died. After 1 year, 34% of the seedlings had died, but none tested positive for *Ceratocystis*, showing that seedlings were not dying due to ROD infections despite the finding of viable *Ceratocystis lukuohia* propagules in the soil. In contrast, unweeded seedlings were six times more likely to suffer mortality than weeded seedlings, and unfenced seedlings were over three times more likely to suffer mortality compared to fenced seedlings.

Because the Yelenik et al. (2020) study was limited to one site and 1 year, seedlings were planted in additional experimental sites in forest reserves with active ROD mortality. Weeding and/or fencing treatments were implemented in these new sites as well. In addition, other planted 'ōhi'a sites in forests that have lower incidence of ROD mortality have been established. While results are still forthcoming, the project has yet to have a single 'ōhi'a seedling test positive for ROD-causing fungi over 3 years of study, from any of the five planting sites (Yelenik, US Department of Agriculture Forest Service, written communication, 2022). While longer-term data will provide further insight, this work substantiates the idea that planting 'ōhi'a, even in forests with high levels of ROD mortality, can successfully increase 'ōhi'a abundance.

12. Disease resistance

Another long-term strategy for returning disease-affected forests is to develop disease resistance lineages and utilize them for restoration efforts (Sniezko, 2006). Work to develop ROD-resistant genotypes of 'ōhi'a was started by the US Department of Agriculture, Agricultural Research Service, and the University of Hawai'i at Hilo in 2016 at the Pacific Basin Agricultural Research Center, where plants of four varieties of *M. polymorpha* (var. *incana*, var. *newellii*, var. *glaberrima*, and var. *polymorpha*) were inoculated with *C. lukuohia*. About 5% of the plants in this study survived this inoculation, with var. *incana* and var. *newellii* showing more resistance (Luiz et al., 2021), although the sample size was too small to reach an estimate of prevalence of resistance in 'ōhi'a populations at large. A much larger program to screen for genetic resistance is now underway at the US Department of Agriculture Forest Service Institute of Pacific Islands Forestry, focusing on collecting genetic material from potentially resistant trees and screening germplasm from across the state (Luiz et al., 2022). Researchers are collecting and rooting cuttings from healthy trees in forests with high levels of disease in hopes that some of these might be disease resistant trees rather than just escapes. These plants will be tested using inoculation trials. Seeds from all the native Hawaiian taxa of *Metrosideros* are also being raised for future inoculation and resistance screening. Researchers plan to outplant resistant trees from the trials into seed orchards to facilitate seed collection for future restoration projects. Although resistant trees would be an important part of an overall recovery program, the scale of forest loss due to ROD far outstrips Hawai'i's ability to restore forests. Protecting the remaining healthy forests is the most effective management tool for ROD.

13. Future activities

It has only been 7 years since ROD was first identified in Hawai'i, and much has been learned about the diseases since then. While proving pathogenicity, describing and naming the two new *Ceratocystis* species, and developing early and accurate detection and identification methods have given us a better understanding of the ROD pathogen biology and disease cycle, these were only the first steps to beginning to unravel how the *Ceratocystis* species infect trees, how they move from tree to tree and throughout the landscape, and how they can best be managed. Many questions remain unanswered, including the following: Where exactly did the ROD pathogens come from and how did they get to Hawai'i? We know that wounding is necessary for pathogen entry, but how long do wounds remain vulnerable to infection? What are the key environmental factors that influence ROD disease susceptibility and disease progression in wild populations of 'ōhi'a? What role does ROD-infected soil or water play for new infections? Is local spread happening or are new infection sources being introduced and how? Are there biological control options for ROD? Are there other host species that are vulnerable to *C. lukuohia* and *C. huliohia*? What role are ambrosia beetles playing in direct disease spread? Can lowering frass production lower the levels of ROD infection? And finally, can ROD be detected in a tree before visible symptoms are observed? Collaborative efforts between researchers, extension agents, and land managers can help provide answers to these questions with additional research.

The information presented above has been useful to managers in developing integrated management program for ROD; however, it is still too early to tell how much of the 'ōhi'a forest will be killed by ROD and how much can be saved.

There is still a lot that we do not know about ROD. However, among the most important activities that could be continued would be the identification of recently dead 'ōhi'a trees that were killed by ROD and the treatment of these trees to limit spread of frass, along with protecting intact forests from damage by feral animals. Additional capability to detect ROD-killed trees and determining the most cost-effective means to treat infected trees will be critical to the success of this effort.

14. Conclusions

Rapid 'Ōhi'a Death is associated with an important pair of diseases (Table 15.1). These have demonstrated a capacity to rapidly kill large numbers of an important tree species in Hawai'i and were unknown 7 years ago. The etiology of the diseases has been described (Barnes et al., 2018; Hughes et al., 2020; Juzwik et al., 2019; Keith et al., 2015). This discovery was made possible by the development and diligent use of appropriate molecular genetic techniques to determine genetic differences between species, strains, and even isolates of these fungi. Some of these same tools have also been useful for determining where and how these fungi have been spreading in Hawai'i. The evidence is strong that movement is taking place in random episodic events.

Extensive efforts have been made to control the spread of these diseases, and no major storms have occurred in Hawai'i since 2018. At the time of this writing, it appears that both of these factors may have had some beneficial effect. However, as *C. lukuohia*, the more devastating of the two fungi involved with ROD, may benefit from severe hurricane conditions causing large amounts of wounding it may take until the aftermath following the next major hurricane that strikes the Hawaiian

TABLE 15.1 Key characteristics of the two diseases associated with the Rapid 'Ōhi'a Death fungal pathogens.

Key characteristics	<i>C. lukuohia</i>	<i>C. huliohia</i>
External symptoms	Rapid browning and wilt of entire crown in several weeks to several months	Decline and dieback of individual limbs that can progress to affect entire crown over months to years
Internal symptoms	Dark sapwood discoloration appearing initially as longitudinal spots and streaks that can progress to completely stain the circumference of outer limb and/or stem sapwood. In cross section, dark staining occurs in distinct radial streaks in the outer sapwood around part or the entire circumference of the stem and/or limb. Staining often found throughout sapwood of infected trees	Well-defined cankers found in inner phloem and cambium underlain by similar shaped stained sapwood. In cross section, diffuse or amorphous dark gray staining in sapwood. Staining may occur in a distinct pie-shaped pattern or have more diffuse borders. Sapwood staining associated with well-defined cankers on stems and limbs
External signs	Fungal perithecia on exposed wood tissue (rare)	Fungal perithecia on exposed wood tissue (rare)
Disease classification	Systemic vascular wilt	Canker disease

Islands to determine the future of these diseases in Hawai‘i. In the meantime, several actions can be taken to help reduce the chances of more ‘ōhi‘a trees being killed by ROD. Important beneficial actions that could be taken include (1) finding dying or recently dead ‘ōhi‘a trees producing copious amounts of infective frass and treating these; (2) raising public awareness to educate people of the importance of not wounding ‘ōhi‘a and of preventing the human-aided distribution of the fungus; and (3) fencing priority conservation forests to protect them from feral cattle, sheep, pigs, and goats so they cannot wound the boles or root systems of ‘ōhi‘a trees.

To date, almost all of the ‘ōhi‘a mortality has been on Hawai‘i Island but the diseases have also been confirmed on the islands of Kaua‘i, Maui, and O‘ahu. Monitoring and management of these new infections may allow local land managers to control ROD outbreaks before they spread widely.

15. Exercises or study questions

1. Can you draw the life cycle of Rapid ‘Ōhi‘a Death on the ‘ōhi‘a tree?
2. What things could be done to make ‘ōhi‘a trees less vulnerable to wounding?
3. How are ambrosia beetles potentially contributing to the spread of ROD?
4. How have Hawaii’s ecosystems and culture been impacted to date and how will these be affected if even more ‘ōhi‘a trees are lost to ROD?
5. What has Hawaii’s Forestry Extension team done to galvanize the public to protect the ‘ōhi‘a tree from ROD?
6. How have molecular genetic techniques been used to discover the exact pathogen involved in causing ROD?
7. What would you do if you went to a new place and found that lots of trees were dying from something you did not know about?

Disclaimer

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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