Abundance of ʻŌhiʻa-Associated Ambrosia Beetles in Two Sites with Rapid ʻŌhiʻa Death Outbreaks

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Abstract. ʻŌhiʻa lehua (*Metrosideros polymorpha* Gaudich.) is the dominant tree in native Hawaiian forests but is threatened by two pathogenic fungi (*Ceratocystis* spp.) which cause Rapid ʻŌhiʻa Death (ROD). Understanding the spread of ROD is vital to informing prevention and management strategies. Ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) contribute to the spread of disease by releasing contaminated frass into the environment or carrying fungal spores between trees on their bodies. We quantified the abundance of ʻōhiʻa-associated ambrosia beetles and their potential contributions to fungal spread within two study sites experiencing active ROD outbreaks. We established a grid of beetle traps at each site, cultured trap samples for viable *Ceratocystis*, and compared the spatial distribution of beetle captures with that of ʻōhiʻa trees showing symptoms of ROD. Nearly all captured ʻōhiʻa-associated beetles were *Xyleborinus saxesenii* (Ratzeburg) or *Xylosandrus crassiusculus* (Motschulsky), both introduced species that utilize many plant hosts. For both species, abundance was unrelated to distance to the nearest symptomatic ʻōhiʻa tree. However, at one of our sites, *Xylosandrus crassiusculus* abundance was higher on one side of a fence line, where there were more symptomatic ʻōhiʻa within a denser and more diverse forest. Culturing the collected samples (beetles, water, and debris) produced instances of *Ceratocystis* viability in samples both with and without ʻōhiʻa-associated beetles, supporting the potential for transmission via frass carried by wind as well as direct transmission by beetles. The community of ʻōhiʻa-associated beetles we captured differed from previous findings at lower elevation sites, highlighting the complexity of beetle-mediated fungal infection risk.

Keywords: Scolytinae, *Xyleborinus saxesenii, Xylosandrus crassiusculus*, forest pests, invasive species, *Ceratocystis*

ʻŌhiʻa lehua (*Metrosideros polymorpha* Gaudich.) is the most common native tree species in Hawaiian forests in terms of both numbers of individuals and biomass, and thrives across a large climatic gradient (Friday and Herbert 2006, Owen et al. 2022). ʻŌhiʻa provide critical habitat for many native Hawaiian species, including endemic and critically endangered birds, arthropods, and snails, and are central to native Hawaiian culture and identity (Jacobi et al. 2024, Mueller-Dombois et al. 2013, Roy et al. 2024a). Recently, ʻōhiʻa trees have been threatened by a pair of fungal diseases, collectively referred to as Rapid ʻŌhiʻa Death (ROD). ROD was first identified in 2014 in the district of Puna on the Island of Hawaiʻi (Keith et al. 2015). The two fungal species responsible for the disease have been identified as *Ceratocystis lukuohia* and *Ceratocystis huliohia* (Barnes et al. 2018), with the former being more virulent. Once established in the xylem of the tree, the fungus spreads within this vascular tissue, and can ultimately occlude it, leading to tree death. A change in leaf color is an important indicator of ROD infection and is used to track the spread of the disease across the landscape (Asner et al. 2018, Perroy et al. 2021, 2020). More than one million trees are estimated to have died from ROD, primarily on the island of Hawaiʻi (Cannon et al. 2022). As ROD continues to threaten ʻōhiʻa, understanding the causes of the spread of this disease remains critical to managing and mitigating its effects.

Wood-boring ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) can facilitate spread of fungal pathogens that impact forest and nursery trees, sometimes leading to tree death (Ranger et al. 2016, Werle et al. 2015). In the case of ROD, ambrosia beetles expel frass (i.e., wood particles and beetle feces) during gallery construction that can contain viable *Ceratocystis* propagules (Roy et al. 2020, 2019). Frass expelled by ambrosia beetles from ʻōhiʻa trees infected with *Ceratocystis* (hereafter, *Ceratocystis*-positive) into the environment could also inoculate uninfected trees, particularly if they are mechanically wounded by ungulates or wind. The contribution of ambrosia beetles to *Ceratocystis* spread parallels findings from other systems (Roy et al. 2023). For example, *Xyleborus affinis* Eichhoff frass has been identified as a potential dispersal agent of *Ceratocystis fimbriata*, which causes mango wilt in Brazil (Souza et al. 2013). *Ceratocystis platani*, which infects the native Oriental plane tree in Greece, has been found in the frass of the ambrosia beetle species *Platypus cylindrus* Fabricius (Ocasio-Morales et al. 2007). In addition, laboratory studies have shown that ambrosia beetles harboring viable *Ceratocystis* inoculum on their bodies can directly infect ʻōhiʻa seedlings, indicating that beetles could play a role in direct transmission of the disease (Roy et al. 2023).

To date, four non-native (*Xyleborinus saxesenii* Ratzburg, *Xyleborus affinis, Xyleborus ferrugineus* Fabricius, and *Xyleborus perforans* Wollaston) and one native (*Xyleborus simillimus* Perkins) ambrosia beetle species have been definitively associated with *Ceratocystis*-positive ʻōhiʻa, having been reared from bolts with viable fungus and shown to produce frass containing viable inoculum (Roy et al. 2020). *Xyleborinus saxesenii* made up 80% of the beetles reared from *Ceratocystis*-positive ʻōhiʻa on the Island of Hawaiʻi (Roy et al. 2020). One additional non-native species, *Xylosandrus crassiusculus*, was reared from a *Ceratocystis*-positive ʻōhiʻa tree on Kauaʻi, although it was not confirmed that the bolt itself contained viable *Ceratocystis* (Dunkle et al. 2023).

Xylosandrus crassiusculus has not been documented to produce frass containing viable *Ceratocystis*. The ambrosia beetle community has received the most study at low elevation sites, and ambrosia beetle community composition has been found to vary with elevation (Roy et al. 2024b, 2020). Investigating how beetles may contribute to ROD spread is particularly important in high elevation forests, where native biodiversity is concentrated.

Analyses of spatial patterns of ambrosia beetle abundance provide opportunities to assess the role of beetles in the spread of ROD. In particular, a striking pattern that has emerged is that fencing built to exclude ungulates from healthy forests effectively minimizes the prevalence of ROD (Fortini et al. 2019). Sharp differences in the density of ‘ōhi‘a trees affected by ROD have been documented across fence lines because wounds in ‘ōhi‘a caused by ungulates can be an important entry point for the pathogen (Perroy et al. 2021). However, it is unknown whether the abundance of ‘ōhi‘a-associated beetles similarly differs across these fence lines, and whether beetle abundance is consistently higher near infected trees. Because ambrosia beetles can fly over fences, beetles that produce frass and emerge from *Ceratocystis*-positive trees could increase disease risk within fenced management units adjacent to areas with outbreaks of ROD.

This study investigated the relationship between ambrosia beetles and the potential spread of ROD by asking: (1) are ‘ōhi‘a-associated ambrosia beetles more abundant closer to known or suspected *Ceratocystis*-positive trees, and on the side of a fence with a ROD outbreak? and (2) how frequently do samples collected from beetle traps contain viable *Ceratocystis*? To address these questions, we trapped beetles at two sites, including one in which symptomatic trees were concentrated on one side of a fence. The drivers and distribution of ambrosia beetle abundance in the Hawaiian Islands are largely unknown, and we predicted higher beetle abundance in traps closer to trees with ROD symptoms. We tested for a management unit effect within our site separated by a fence line and predicted that a difference in beetle abundance would be observed if beetles are both more abundant within a site experiencing an outbreak and do not frequently disperse across the fence line. Our study provides insight into whether and how beetles emerging from ROD-positive trees could elevate infection risk in surrounding forest.

Methods

Study sites and data collection. Our study sites (Fig. 1) were located in two ‘ōhi‘a-dominated forests on the island of Hawai‘i that had experienced a recent ROD outbreak (Perroy et al. 2021). One study site was in the Koa Unit of the ‘Ōla‘a Forest within Hawai‘i Volcanoes National Park in the ahupua‘a of ‘Ōla‘a in the moku of Puna (coordinates: 19.4599, –155.2455) at approximately 1160 m elevation (hereafter, ‘Ōla‘a). The second study site spanned the Kahuku Unit of Hawai‘i Volcanoes National Park and the adjacent Ka‘ū Forest Reserve in the ahupua‘a of Kahuku in the moku of Ka‘ū (19.0886, –155.6676) at approximately 820 m elevation (hereafter, Kahuku). ‘Ōla‘a is a relatively dense rainforest with a canopy dominated by ‘ōhi‘a, with lesser amounts of ‘ōlapa (*Cheirodendron trigynum*), kāwa‘u (*Ilex anomala*), and kōlea (*Myrsine lessertiana*). The understory is predominantly composed of native hapu‘u tree ferns (*Cibotium* sp.), kanawao (*Broussaisia arguta*), and the invasive kahili ginger (*Hedychium gardnerianum*). ‘Ōla‘a shares a fenced boundary with the ‘Ōla‘a Forest Reserve

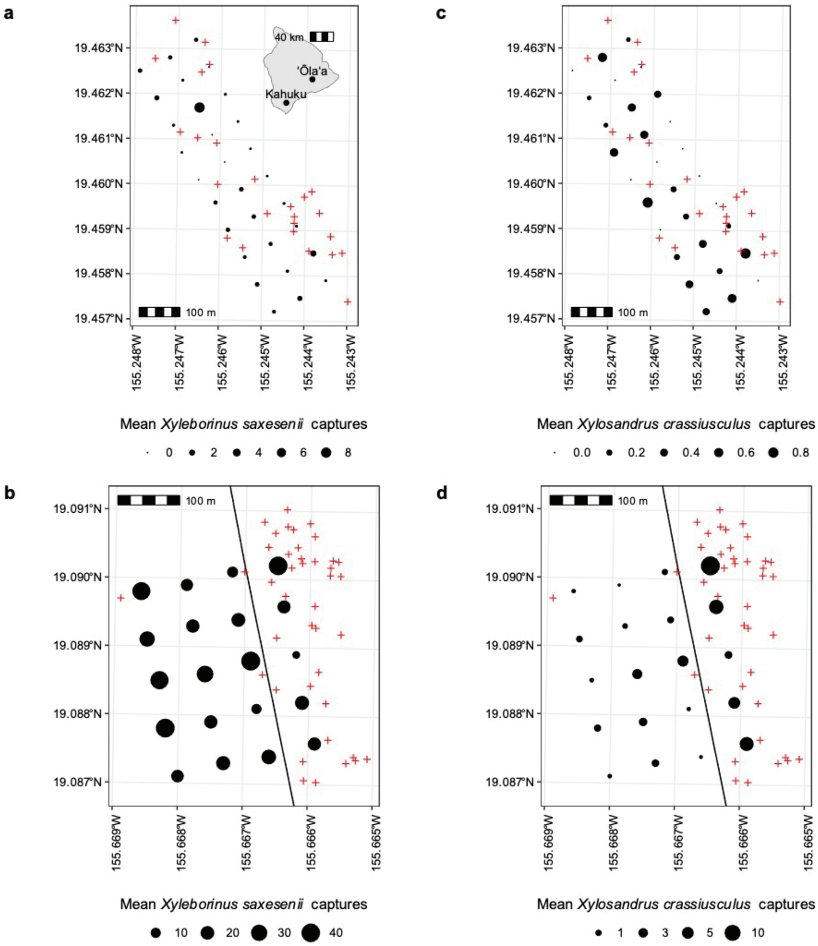

Figure 1. Mean *Xyleborinus saxesenii* captures by trap at (a) ʻŌlaʻa and (b) Kahuku, and mean *Xylosandrus crassiusculus* captures by trap at (c) ʻŌlaʻa and (d) Kahuku. The locations of trees suspected or confirmed to be *Ceratocystis*-positive are shown in all panels with red crosses. The inset in the top right of (a) shows the position of each study site on the island of Hawaiʻi. The line through the Kahuku plots shows the fence location, which separates Hawaiʻi Volcanoes National Park on the west side of the fence from the Kaʻū Forest Reserve on the east side of the fence.

(managed by the Hawaiʻi Department of Land and Natural Resources) and a private ranch that both contain feral pigs and cattle. Our study area in ʻŌlaʻa had been largely free of ungulates until a recent incursion of pigs across a compromised section of fence between 2019 and 2020 (Perroy et al. 2021). The Kahuku study site spans a fenced National Park unit in which ungulates are managed and an unfenced Forest Reserve where ungulates are not managed. Due to a history of cattle ranching, the Kahuku Unit of Hawaiʻi Volcanoes National Park supports a less dense ʻōhiʻa forest with few other tree species contributing to canopy coverage. Its understory is dominated by non-native grasses, along with the native uluhe fern (*Dicranopteris linearis*), invasive sword ferns (*Nephrolepis brownii*), and some kahili ginger. Vegetation is denser in the adjacent Kaʻū Forest Reserve, which includes an understory dominated by hapuʻu tree ferns, more kahili ginger than the fenced side, māmaki (*Pipturus albidus*), and common guava (*Psidium guajava*). Our study area within the Kahuku Unit of Hawaiʻi Volcanoes National Park is largely free of pigs and other ungulates while pigs are numerous in the Kaʻū Forest Reserve. Average wind speed based on interpolated weather station data was estimated at 2.4 m/s for ʻŌlaʻa and 2.8 m/s for Kahuku (Giambelluca et al. 2014); gridded wind direction data are not available.

ʻŌhiʻa trees suspected to be symptomatic of ROD were identified via imagery collected primarily by helicopter and supplemented with satellite data. We looked for symptomatic trees in imagery from the year prior to the start of beetle trapping through the end of the trapping period at each site. We considered imagery from prior to the start of sampling to allow for beetle development within stressed ʻōhiʻa; we expected our traps to capture beetles emerging from trees and searching for new hosts.

Analyses of aerial imagery collected 8/5/2020 found an outbreak of ROD in ʻŌlaʻa (Perroy et al. 2021). Aerial imagery was collected from a helicopter at 800 feet altitude with a Nikon D850 with 35mm lens at 1/2000 second shutter speed, Auto ISO, aperture F5.6, with auto focus. From the imagery, suspect ROD trees were identified and given a confidence rating of being *Ceratocystis*-positive following Perroy et al. (2021). Within the boundary of the ʻŌlaʻa study area, 23 trees with foliage that indicated infection due to ROD were detected (Fig. 1a). Of these, 21 were classified as highly suspect and 2 were classified with medium confidence. Ground sampling by the National Park Service tested 11 of these suspect trees, including 10 highly suspect trees and 1 medium confidence tree; all of these were confirmed to be positive for *Ceratocystis* DNA via quantitative polymerase chain reaction (qPCR) conducted at the U.S. Department of Agriculture U.S. Pacific Basin Agricultural Research Center (Heller and Keith 2018). Therefore, our analyses assumed that all suspect trees classified with high or medium confidence were *Ceratocystis*-positive. In addition, two suspect trees in ʻŌlaʻa were identified from satellite imagery collected 1/14/2021. Because in the ʻŌlaʻa site we observed many standing, recently dead trees, aerial imagery from 8/5/2021 and 9/15/2021 was analyzed to identify the locations of 135 such trees (Fig. 2). These additional trees retained fine white branches, which established the recency of their death, but did not meet the high or medium confidence classification criteria for suspected *Ceratocystis*. These trees, hereafter referred to as ʻfine white,ʼ were not tested for *Ceratocystis*.

Aerial imagery at the Kahuku study site was collected in December 2020 using the above helicopter-mounted camera specifications and similarly processed to identify 31 suspected *Ceratocystis*-positive trees. Suspected *Ceratocystis*-positive trees occurred at a higher density within the Kaʻū Forest Reserve portion of our study site, compared to the National Park unit to the west of the fence (Fig. 1b). Additional satellite-derived suspect trees were recorded from imagery collected on 1/22/2021 (n = 2 trees), 5/30/2021 (n = 4 trees), and 12/2/2021 (n = 4 trees).

Panel traps (Multitrap Black Panel Trap with narrow panels, Synergy

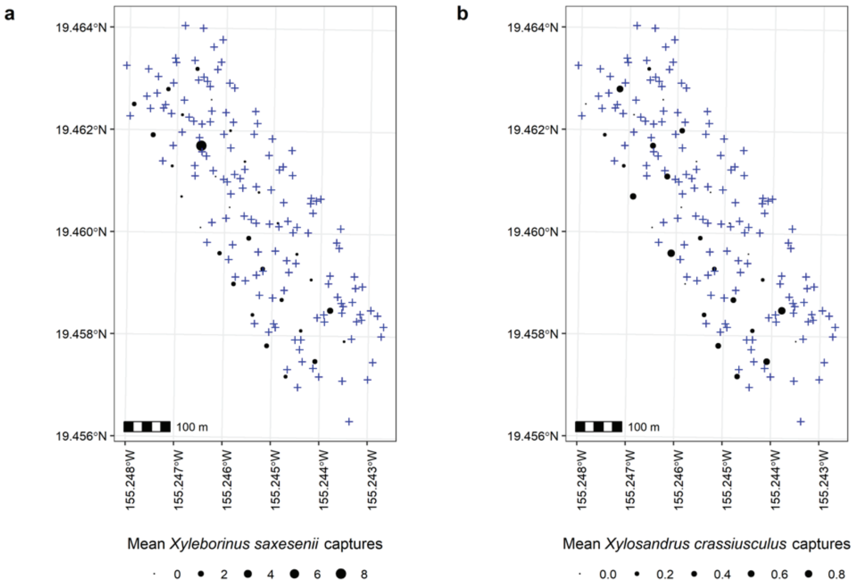

Figure 2. Mean (a) *Xyleborinus saxesenii* and (b) *Xylosandrus crassiusculus* captures at each trap location in ‘Ōla‘a. The location of dead ‘ōhi‘a trees with fine white branches is shown in blue crosses.

Semiochemicals Corp.) were used to collect ambrosia beetles and *Ceratocystis* inoculum in both study sites. Thirty traps were deployed in ‘Ōla‘a and 20 were established in Kahuku. In both sites the traps were arranged in a grid with traps 75 m x 75 m apart within the forest (10 x 3 at ‘Ōla‘a and 5 x 4 at Kahuku). At Kahuku one line of four traps was located in the Ka‘ū Forest Reserve, with the goal of testing whether ‘ōhi‘a-associated beetles were more abundant within the Ka‘ū Forest Reserve compared to the adjacent Kahuku Unit of Hawai‘i Volcanoes National Park, and evaluating whether there was a pattern of decreasing beetle numbers with increasing distance from symptomatic trees. We predicted that if ‘ōhi‘a-associated beetles frequently disperse from the Forest Reserve to the National Park, we would observe a gradient of abundance with increasing distance from the Forest Reserve. Traps were hung from branches or suspended between two trees using paracord; the bottom of the trap hung approximately 1 m off the ground. Beetles were collected in a cup attached to the bottom of the trap containing approximately 50 mL of distilled water; collecting in water enabled culturing for fungal viability. Traps were hung under 1.5-m (60-inch) diameter umbrellas to shield them from rain and prevent overflow of water cups that would otherwise occur in wet forests (refer to photo in Roy et al., 2024b). Traps were set with 50 mL of a lure within a 50-mL conical plastic centrifuge tube. An approximately 1-cm diameter hole was drilled in the cap of each tube to allow release of the lure. The panels of the traps were wiped down with isopropyl alcohol at the time of sample collection to sanitize the traps and prevent contamination from previous weeks.

Samples from ‘Ōla‘a were collected weekly between April and November 2021. Before June 15, 2021, the lure in each trap was rotated between three different lures: 100% ethanol, an approximately 50:50 mixture of ethanol and methanol (Klean-Strip® Denatured Alcohol W.M. Barr & Co., Inc., Memphis, Tennessee), and deionized water. After June 15, 2021, traps either contained ethanol as a lure or water as a control because ethanol was found to be more effective than the ethanol:methanol mixture (Roy et al. 2024b). The purpose of the water lure was to act as a control for the fungal viability portion of the study; traps baited with water were expected to catch far fewer beetles, and samples without any ‘ōhi‘a-associated ambrosia beetles would allow us to estimate the frequency with which wind carries viable inoculum, whereas viability in samples with beetles could be attributed to wind or could have been carried by the beetles. At Kahuku, we began with two weeks of trapping with ethanol baits in November 2021, during which samples were not cultured for fungal viability. This was followed by two 4-week sets of weekly sampling for a total of 10 weeks of trapping between November 2021 to February 2022. In each of the 4-week sampling sets, traps used ethanol as a lure and water as a control, and the water lure was cycled through each trap position. Occasionally traps fell during the collection period, and data from those collections were excluded from analyses.

Patterns of beetle abundance. The beetles in each sample were identified to the species level in accordance with Samuelson’s (1981) dichotomous key and compared to reference collections including those held at the Bernice Pauahi Bishop Museum. Given the strong morphological similarity between *Xyleborus dubiosus* and *Xyleborus simillimus*, a subset of individuals were DNA barcoded. DNA was extracted with the DNeasy Blood and tissue Kit (Qiagen, Carlsbad, CA) and run through a polymerase chain reaction (PCR) with mitochondrial cytochrome c oxidase I (COI) primers before being sent for sequencing at Genewiz (Azenta Inc., La Jolla, CA). Resulting sequence ends were manually trimmed and reverse aligned using Geneious 8.1.9 software (Auckland, NZ) and then compared to publicly available COI sequence data using the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov/Genbank) where the sequences were then submitted (Accession numbers: PV335703-PV335710). Our analyses focused on beetle species documented to use ‘ōhi‘a, of which two species, *Xyleborinus saxesenii* and *Xylosandrus crassiusculus*, were captured in sufficient abundance to analyze. For patterns of abundance, we considered only traps with an ethanol lure, as ethanol is the most effective lure (Montgomery and Wargo, 1983; Roy et al., 2024b).

To examine local spatial patterns, we tested if the distance from each trap to the closest suspected *Ceratocystis*-positive tree affected the abundance of each ‘ōhi‘a-associated ambrosia beetle species captured. We fit generalized linear mixed models assuming a negative binomial distribution, where the response was the count of either *Xyleborinus saxesenii* or *Xylosandrus crassiusculus* in each sample. A separate model was fit for each species at each site. All models included a fixed effect of the distance to the nearest *Ceratocystis*-positive tree. For the Kahuku site, we included a management effect that tested for a difference between traps on either side of the fence line that separated the National Park unit (16 traps; n = 113 ethanol-baited samples) from the Forest Reserve (4 traps; n = 35 ethanol-baited samples). Models included random effects of trap ID and date to account for the lack of independence between multiple samples collected from the same place and multiple samples collected at the same time. We estimated models using the glmmTMB package in R (Brooks et al. 2017) and checked model diagnostics using the DHARMa package (Hartig 2022). We tested for zero-inflation, confirming that our observed data had a proportion of zeros that would be expected for count data with the estimated parameters. To visually relate within-plot patterns of beetle captures to the presence of nearby *Ceratocystis*-positive trees, the mean number of beetles captured per trap was calculated and plotted alongside the *Ceratocystis*-positive tree locations.

Within ʻŌlaʻa there were additional recently dead trees, the ʻfine whiteʼ trees described above. Because beetles are likely to attack trees that are stressed or dying for reasons aside from ROD, we also asked whether the beetle capture at each trap was related to the distance to the nearest ʻfine whiteʼ ʻōhiʻa tree, fitting the same generalized linear mixed model described above using distance to ʻfine whiteʼ rather than suspected *Ceratocystis*-positive tree locations.

***Ceratocystis* viability in samples collected from panel traps.** Fungal viability was assessed in traps baited with either a water or an ethanol lure beginning in June 2021. Traps with a water lure were cultured to assess the rate at which viable fungal spores were carried by wind, as few beetles were expected to be captured in the water-baited traps. Beetles were removed for identification, and afterwards the collected sample, including the beetles, water, and any debris caught in the trap, was cultured to check for *Ceratocystis* presence following Roy et al. (2023). Carrot slices were inserted into the samples to provide a growth medium for *Ceratocystis*. The carrots were surface sterilized with 70% isopropyl alcohol, rinsed with deionized water, and then soaked in a 10% streptomycin antibiotic solution prior to culturing to prevent contamination. The samples were then hooked up to a water aeration machine that ensured the fungus had enough oxygen present in the water to grow. The samples were aerated for two weeks and checked for fungal presence. For samples that appeared to have fungal growth indicative of *Ceratocystis* such as mycelial or perithecial structures, qPCR was used to verify the presence of *Ceratocystis,* and the *Ceratocystis* was identified as either *C. huliohia* or *C. lukuohia*. We calculated the percentage of samples in which viable *Ceratocystis* was detected and plotted the locations of the traps producing these samples alongside the locations of *Ceratocystis*-positive ʻōhiʻa.

Spatial data management, analyses, and visualizations were done in R version 4.2.2 (R Core Team 2022) using the tidyverse (Wickham et al. 2019) and sf (Pebesma and Bivand 2023) packages.

Results

Patterns of beetle abundance. In ʻŌlaʻa, the most abundant ʻōhiʻa-associated species was *Xyleborinus saxesenii*, of which we captured 239 individuals (24% of total ambrosia beetle captures with an ethanol lure at this site). *Xylosandrus crassiusculus* (n = 65; 6% of total) was also captured. Capture rates were an order of magnitude higher with the ethanol lure compared to a water lure (Table 1), and so our analysis focuses on ethanol-baited traps. Other captured species not known to use ʻōhiʻa included the native species *Xyleborus dubiosus* (n = 700 individuals; 69% of total) and *Xyleborus pele* (n = 12; 1% of total). Sequenced individuals were confirmed to be *Xyleborus dubiosus* with a 98% or greater query match to NCBI databases. Of the 292 samples (each representing a single trap with an ethanol lure over a one-week period, excluding traps that fell), 90 samples (31%) contained no ambrosia beetles. *Xyleborinus saxesenii* were captured in 52 samples (18% of samples). When *Xyleborinus saxesenii* were captured, the most frequent number of individuals in an individual trap was 1 beetle (n = 20 samples), but we collected one sample containing 86 *Xyleborinus saxesenii* individuals. For *Xylosandrus crassiusculus,* one individual was the modal trap capture, and up to four individuals were captured in a single trap sample.

Ambrosia beetle abundance was far higher in Kahuku than in ‘Ōla‘a, and again was an order of magnitude higher in traps with an ethanol lure (Table 1). In Kahuku, *Xyleborinus saxesenii* was the species caught in the highest abundance (n = 3488 individuals, 89% of all individuals captured with an ethanol lure) followed by *Xylosandrus crassiusculus* (n = 400, 10%). A single individual each of the native *Xyleborus simillimus*, non-native *Xyleborus affinis*, and non-native *Xyleborus perforans* was captured in an ethanol-baited trap over the entire sampling period; these were the only other taxa captured that have been previously associated with ‘ōhi‘a. Species not known to use ‘ōhi‘a as a host included native *Xyleborus dubiosus* (n = 26, <1%) and native *Xyleborus tantalus* (n = 1, <1%). Of the 148 samples, 27 samples (18%) contained no ambrosia beetles. *Xyleborinus saxesenii* were captured in 111 samples (75%), and although one beetle remained the modal catch number (n = 15 samples), many traps had higher captures, with a maximum of 218 *Xyleborinus saxesenii* individuals in a single sample. For *Xylosandrus crassiusculus*, one was the modal catch number (n = 22 samples), and up to 40 individuals were collected in a single sample.

The number of beetles captured was not related to the distance to the nearest known or suspected *Ceratocystis*-positive tree for either species (Table 2). At ‘Ōla‘a, neither focal beetle species showed a relationship between its captures and the distance to the nearest ‘ōhi‘a tree in the fine white canopy phase (*Xyleborinus saxesenii:* $\beta = -0.01 \pm 0.02$, z = –0.59, $P = 0.56$; *Xylosandrus crassiusculus*: $\beta = 0.004 \pm 0.02$, z = 0.02, $P = 0.99$). At Kahuku, *Xyleborinus saxesenii* abundance was not different between traps in the Forest Reserve versus the National Park, while *Xylosandrus crassiusculus* abundance was lower in traps in the National Park compared to the Forest Reserve (Tables 1 and 2). Random effect estimates indicated that trap explained less variation in the data than date, i.e., on some sampling dates captures were generally high or low across traps (Table 2). The exception was for *Xylosandrus crassiusculus* at Kahuku, where differences among traps were of a similar magnitude as differences among dates.

***Ceratocystis* viability in samples collected from panel traps.** In ‘Ōla‘a, 5 of 342 (1.5%) trap samples contained viable fungal spores (Fig. 3a), all of which were identified as *C. lukuohia*. Of the five viable samples, four were collected from the same trap (twice in June and once each in July and October). One of the viable samples was from a sampling period in which the trap was set with a water lure, while the other viable samples were from ethanol-baited sampling periods. The July sample contained a single *Xyleborinus saxesenii*, while the other infectious samples from that trap did not contain any ‘ōhi‘a-associated beetles. One other trap yielded one sample containing viable *Ceratocystis*, which did not contain any ‘ōhi‘a-associated beetles. Of cultured samples collected in ‘Ōla‘a, 4 out of 303 samples that did not contain ‘ōhi‘a-

Table 1. Average number of individuals captured per trapping occasion for our two focal species, *Xyleborinus saxesenii* and *Xylosandrus crassiusculus*. Our analysis of abundance focused on ethanol-baited traps because an order of magnitude more beetles were captured in traps baited with ethanol compared to traps baited with water. Both species were more abundant at Kahuku than at ʻŌlaʻa, and within Kahuku, our analysis tested for a difference in abundance across the fence line separating Hawaiʻi Volcanoes National Park (Kahuku-HAVO) from the Kaʻū Forest Reserve (Kahuku-FR). The percentage of samples that contained viable *Ceratocystis* suggested that wind-borne frass contributed to viability; this was most evident in ʻŌlaʻa where samples were less likely to contain ʻōhiʻa-associated ambrosia beetles.

Site and Management	Lure	***Xyleborinus saxesenii*** **mean count (sum of captures / number of trapping occasions)**	***Xylosandrus crassiusculus*** **mean count (sum of captures / number of trapping occasions)**	**Percent viability in samples without ʻōhiʻa-associated ambrosia beetles (number positive / number cultured)**	**Percent viability in samples containing ʻōhiʻa-associated ambrosia beetles (number positive / number cultured)**
ʻŌlaʻa	Ethanol	0.82 (239/292)	0.22 (65/292)	1.55 % (3/193)	2.63 % (1/38)
ʻŌlaʻa	Water	0.02 (3/176)	0.03 (5/176)	0.91 % (1/110)	0 (0/1)
Kahuku-HAVO	Ethanol	24.76 (2798/113)	1.35 (153/113)	0 (0/25)	3.33 % (2/60)
Kahuku-HAVO	Water	4.14 (116/28)	0.36 (10/28)	0 (0/24)	0 (0/4)
Kahuku-FR	Ethanol	19.71 (690/35)	7.06 (247/35)	0 (0/3)	3.85 % (1/26)
Kahuku-FR	Water	2.2 (22/10)	0.4 (4/10)	0 (0/7)	0 (0/3)

associated beetles were viable (1.3%) and 1 of 39 samples that did contain ʻōhiʻa-associated beetles was viable (2.6%).

Of the samples collected in Kahuku and cultured, 3 of 152 (2% of samples) contained viable *C. lukuohia* spores. Each of these was collected in a different trap (Fig. 3b) and on a different date, during a sampling period in which the trap was baited with ethanol. In each case the sample contained both *Xyleborinus saxesenii* (n = 26; n = 32; n = 81 individuals) and *Xylosandrus crassiusculus* (n = 5; n = 8; n = 2 individuals). The samples in which an individual of *Xyleborus simillimus, Xyleborus affinis,* or *Xyleborus perforans* was captured did not contain viable *Ceratocystis* spores. Of cultured samples collected in Kahuku, none of the 59 samples that did not contain ʻōhiʻa-

Table 2. Generalized linear mixed models testing for a relationship between distance to the nearest known or suspected *Ceratocystis*-positive ʻōhiʻa lehua (*Metrosideros polymorpha* Gaudich.) tree and beetle abundance found no association. Models for Kahuku included a management effect to differentiate between traps on National Park Service and Forest Reserve lands; the Forest Reserve was the baseline and models estimated the difference in the National Park Unit. We caught more *Xylosandrus crassiusculus* in traps within the Forest Reserve at Kahuku. The estimated standard deviation of the trap and date random effects are presented; there was more variation among dates than among traps except for *Xylosandrus crassiusculus* at Kahuku.

	***Xyleborinus saxesenii* at ʻŌlaʻa**			***Xyleborinus saxesenii* at Kahuku**			***Xylosandrus crassiusculus* at ʻŌlaʻa**			***Xylosandrus crassiusculus* at Kahuku**		
Parameter	Estimate	z value	*P* value	Estimate	z value	*P* value	Estimate	z value	*P* value	Estimate	z value	*P* value
Intercept	–2.29 ± 0.72	–3.18	0.001	2.72 ± 0.50	5.44	<0.0001	–2.75 ± 0.61	–4.53	<0.0001	1.87 ± 0.44	4.25	<0.0001
Distance to nearest suspect tree (m)	0.007 ± 0.006	1.23	0.22	–0.001 ± 0.003	–0.54	0.59	0.007 ± 0.006	1.12	0.26	–0.003 ± 0.004	–0.61	0.54
Management unit	-			–0.08 ± 0.34	–0.24	0.81	-			–1.78 ± 0.54	–3.32	0.0009
Random effect of trap	0.6			0			0.9			0.7		
Random effect of date	2.3			1.3			1.4			0.6		

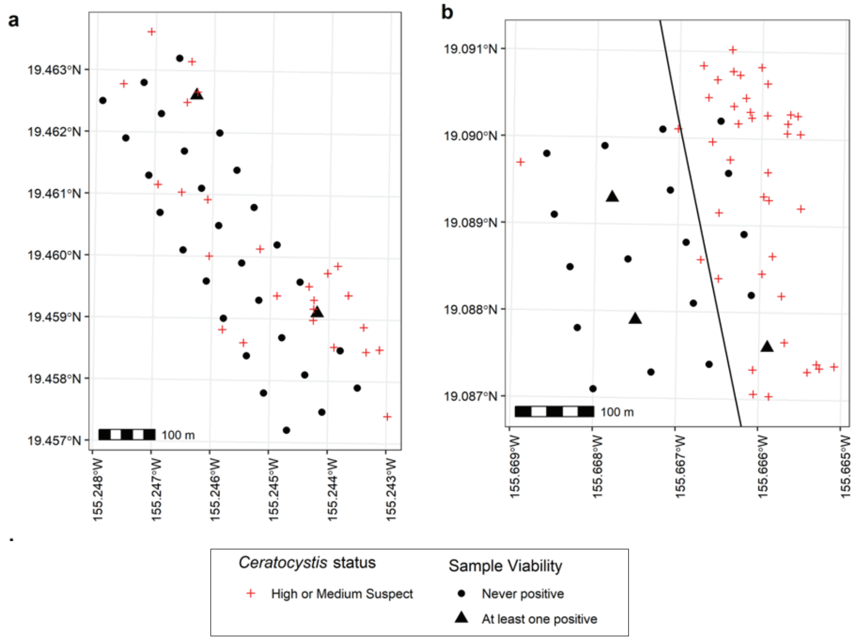

Figure 3. History of sample viability for each trap's location, with location of trees suspected to be *Ceratocystis* positive. The line through the Kahuku plot shows the fence location, which separates Hawai'i Volcanoes National Park on the west side of the fence from the Ka'ū Forest Reserve on the east side of the fence.

associated beetles were viable (0%) and 3 of 93 samples that did contain 'ōhi'a-associated beetles were viable (3.2%). Across both sites, the frequency of detecting viable *Ceratocystis* in samples that did not contain 'ōhi'a-associated beetles (4 viable of 362 samples) and that contained at least one individual of an 'ōhi'a-associated beetle species (4 viable of 132 samples) was not statistically different (Chi-squared test with simulated *P* values because of small expected counts: $\chi^2 = 2.25$, $P = 0.22$).

All data are publicly available at https://doi.org/10.5066/P14GSCLY (Dunkle et al. 2025).

Discussion

The beetle community at our study sites was dominated by a subset of the invasive species associated with *Ceratocystis*-positive 'ōhi'a at lower elevation sites. *Xyleborinus saxesenii* was the most abundant 'ōhi'a-associated beetle caught in traps, consistent with previous findings that it was the species most frequently reared from *Ceratocystis*-positive 'ōhi'a (Roy et al. 2020). Beetle abundance was substantially higher at Kahuku compared to 'Ōla'a (Table 1). Ambrosia beetle captures were highly variable within sites, but at Kahuku we found that *Xylosandrus crassiusculus* was more abundant within the Ka'ū Forest Reserve, compared with the adjacent unit of Hawai'i Volcanoes National Park. This pattern indicates that small-scale variation in host availability and/or forest structure can underlie differences in beetle abundance. Nevertheless, we did not capture more 'ōhi'a-associated beetles at traps closer to symptomatic 'ōhi'a trees.

We cultured viable fungal spores from traps, including from samples that did not contain ʻōhiʻa-associated beetles, supporting the potential for wind to carry viable inoculum.

Xyleborinus saxesenii comprised the vast majority of beetles caught at Kahuku, and it was the second-most abundant species at ʻŌlaʻa. *Xyleborinus saxesenii* is a generalist species native to Eurasia that has invaded Oceania, North America, South America, and Africa (Gómez et al. 2013). It generally attacks stressed host trees and can be an important agricultural pest (Saruhan and Akyol 2012). Our study aligns with results from North America that found *Xyleborinus saxesenii* was the most abundant beetle captured in ethanol-baited traps (Rabaglia et al. 2019, Steininger et al. 2015). *Xyleborinus saxesenii* was also the most abundant species elsewhere on the island of Hawaiʻi, comprising 80% of individuals reared from ʻōhiʻa bolts from five sites (Roy et al. 2020). Patterns of *Xyleborinus saxesenii* captures showed that abundance was much higher at Kahuku compared to ʻŌlaʻa, and within each site we observed more variation in abundance across sampling dates than consistent differences in abundance between traps. *Xylosandrus crassiusculus* is also an invasive generalist species documented to attack ʻōhiʻa (Samuelson 1981), originating from Asia, with a large global distribution and well-documented impacts to horticulture and agriculture (Gugliuzzo et al, 2021). Its role in the ROD pathosystem is unknown, as it has been reared from *Ceratocystis*-positive ʻōhiʻa trees on Kauaʻi (Dunkle et al. 2023) but not reared from infected ʻōhiʻa on the island of Hawaiʻi and as such its frass was not tested for viable inoculum (Roy et al. 2020).

Previous work has yielded a more diverse ʻōhiʻa-associated community than our study, reflecting variability in beetle abundance between sites and collection methods. *Xyleborus ferrugineus* was the most abundant species emerging from ROD-positive ʻōhiʻa in one study (Roy et al. 2019) but was not captured in this study. For three other species that have been studied for their role in the spread of ROD (Roy et al. 2020), *Xyleborus affinis, Xyleborus simillimus*, and *Xyleborus perforans,* only a single individual of each species was captured. *Xyleborus simillimus* is a native ambrosia beetle restricted to ʻōhiʻa (Samuelson 1981). Understanding the distribution, abundance, and chemical responses of this species could inform management in any high elevation forests where it is more abundant; it was not detected in other work at low elevation sites with a high prevalence of ROD (Roy et al. 2020, 2019).

We explained within-site variation in abundance only for *Xylosandrus crassiusculus* in Kahuku, where it was more abundant in the Forest Reserve than in the National Park unit on the other side of the fence line. This fence line effect could be due to differences in host availability, including differences in the density of symptomatic ʻōhiʻa trees and/or to differences in the availability of other hosts. The density of symptomatic tress was much higher within the Forest Reserve (Fig. 1), likely due to the prevalence of ungulates (Perroy et al. 2021). The Forest Reserve side of the fence at Kahuku also had a denser forest structure, with a more diverse woody understory that could provide greater abundance and diversity of hosts for generalist non-native beetles. After accounting for differences between management units, variability in captures within sites was not related to spatial patterns of symptomatic ʻōhiʻa trees. Nevertheless, our results demonstrate that beetle communities can show fine scale spatial structure in relation to forest structure and host availability.

Although the proportion of trap samples that tested positive for *Ceratocystis* was low (1–2%) at both sites, our results show that infected inoculum is aerially transported across the landscape, either by wind or on ambrosia beetles. The fact that four of eight samples from which *C. lukuohia* was cultured did not contain beetles indicates that a substantial proportion of transported inoculum is carried by wind. It is unclear if our use of umbrellas to cover the traps from rain affected our results. Previous work has confirmed the presence of airborne *Ceratocystis* DNA (although not necessarily viable inoculum) within ROD-infected areas as well as at locations kilometers from the nearest known infected tree (Atkinson et al. 2019, Atkinson and Roy 2023, Heller et al. 2023, Roy et al. 2021). Highly sensitive assays can detect minute amounts of *Ceratocystis* DNA and have demonstrated a high prevalence (approximately 40%, averaged across sites) of fungal DNA in air, whereas culturing to confirm viability is more challenging; this points to the importance of further work to evaluate wind as a mechanism for long-distance dispersal (Heller et al. 2023). The rates of viable *Ceratocystis* we documented reflect those not of individual beetles, but rather of the weekly sampling period's total catch. We cultured the entirety of the contents of each trap, and therefore *Ceratocystis* spores could have been carried on the beetles' bodies or could have been introduced to our samples via frass carried by wind. In half the viable samples (4 of 8 samples), one or more *Xyleborinus saxesenii* and *Xylosandrus crassiusculus* were collected in the sample, such that the propagule could have been introduced by the beetle or by wind. All three of the positive samples from Kahuku contained both *Xyleborinus saxesenii* (n = 26, 32, and 81 individuals) and *Xylosandrus crassiusculus* (n = 2, 5, and 8 individuals); two of the positive samples were from the National Park unit and one viable sample was from the Forest Reserve (Fig. 3). At our ʻŌlaʻa study site, we documented four instances in which we detected viable *Ceratocystis* spores within a sample that did not contain any ʻōhiʻa-associated beetles, while the fifth sample contained one *Xyleborinus saxesenii* individual. The samples without any beetles derived from two traps, each of which was within 10 m of a suspected ROD tree. The differences between sites in whether samples with viable spores derived from traps in which beetles were captured could simply reflect the higher abundance of beetles at Kahuku, as fewer traps at that site captured no individuals of ʻōhiʻa-associated beetle species. Even a low frequency of successful dispersal of *Ceratocystis* propagules can play an important role in spreading ROD, particularly because both beetles and frass can be carried in the wind long distances.

Important caveats of our study were that not all *Ceratocystis*-positive trees may have been identified, ʻōhiʻa-associated beetle species may not be equally attracted to the ethanol lure, and not all captured individuals of ʻōhiʻa-associated species may have emerged from ʻōhiʻa trees. These limitations could affect our assessment of the relationship between ambrosia beetle abundance in traps and distance to the nearest known or suspected *Ceratocystis*-positive tree. During field work in ʻŌlaʻa, we noted trees with potential ROD symptoms that were not identified as medium or high confidence suspects by the 2020 helicopter survey. This could have been due to subsequent spread or trees may have died for another reason. Stressed and dying trees are likely to attract ambrosia beetles regardless of the cause of their decline, and so we repeated our analysis using trees that had recently died (i.e., those at the ʻfine whiteʼ stage, which had lost their leaves but still retained fine branches). Our findings were upheld, as no relationship was found between beetle captures and the distance to the nearest recently dead ʻōhiʻa.

Anticipating how beetles may contribute to the spread of ROD depends on the beetle community and on the dispersal of both beetles and frass that carry infective spores. Our study demonstrates how two generalist invasive beetle species can differ in their spatial distributions at small scales; *Xylosandrus crassiusculus* showed a difference within the Kahuku site, whereas *Xyleborinus saxesenii* did not. Flight behavior after emergence is poorly understood, although in general bark and ambrosia beetles can either disperse short distances within the forest canopy (e.g., at least 200 m for *Xylosandrus crassiusculus* [Werle et al. 2015]) or can travel with the wind long distances above the forest canopy; many species can fly for kilometers (Jones et al., 2019). However, despite the potential for relatively long-distance flight, most new bark beetle infestations in other studies were shown to occur within 100 m of a previous infestation (Kautz et al. 2011). Patterns of wind can affect dispersal of both beetles and frass; beetles generally fly downwind and may be able to better orient towards lures with less wind, for example within forests rather than in open areas (Salom and Mclean 1991). Although the flight capabilities of ambrosia beetles imply our entire trapping grid could have been available to each captured individual, the small-scale spatial patterns we observed for *Xylosandrus crassiusculus* in Kahuku indicate that this species typically moves short distances. Differences between *Xyleborinus saxesenii* and *Xylosandrus crassiusculus* in their spatial distributions at Kahuku point to the importance of understanding how the composition of ambrosia beetle communities shifts with forest structure and how ambrosia beetle species differ in their host use and dispersal patterns, which affect the frequency of beetle-mediated spread of *Ceratocystis* via the production of infective frass as well as via direct transmission.

Our results highlight the variability in ambrosia beetle abundance within and between sites. Our Kahuku trapping grid spanned a fence line that separated a Forest Reserve containing ungulates and many suspected *Ceratocystis*-positive trees from a less densely forested National Park unit in which ungulates were controlled and fewer trees were symptomatic for ROD (Perroy et al. 2021). We found differences in abundance across this fence line for one invasive beetle but not another, showing that at least one invasive beetle species varies in abundance over short distances in association with variation in forest structure and/or host availability. We did not observe additional effects of distance to the nearest symptomatic tree for either species. Within the National Park portion of the Kahuku site, neither species showed a clear spatial gradient in abundance and beetles were not more abundant in traps closer to the Forest Reserve (Fig. 1). Our work lends further support to the effectiveness of fencing and ungulate control as beneficial measures for maintaining forest health. Further work to understand the abundance, host associations, olfactory preferences, and dispersal patterns of ambrosia beetles could guide the development of management tools to limit the spread of ROD.

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