Technical Report HCSU-087

ECONOMICAL ENVIRONMENTAL SAMPLER DESIGNS FOR DETECTING AIRBORNE SPREAD OF FUNGI RESPONSIBLE FOR RAPID `OHI`A DEATH

Carter T. Atkinson[1], Kylle Roy[1], and Carolina Granthon[2]

[1]U.S. Geological Survey, Pacific Island Ecosystems Research Center, Kīlauea Field Station, P.O. Box 44, Hawai`i National Park, HI 96718

[2]Hawai`i Cooperative Studies Unit, University of Hawai`i at Hilo, Pacific Aquaculture and Coastal Resources Center (PACRC), P.O. Box 44, Hawai`i National Park, HI 96718

Hawai`i Cooperative Studies Unit
University of Hawai`i at Hilo
200 W. Kawili St.
Hilo, HI 96720
(808) 933-0706

UNIVERSITY of HAWAI'I® HILO

This product was prepared under Cooperative Agreement G15AC00191 for the Pacific Island Ecosystems Research Center of the U.S. Geological Survey.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

We designed two new samplers for monitoring airborne particulates that rely on either natural wind currents (Passive Environmental Sampler) or a battery-operated fan (Active Environmental Sampler). Both samplers are significantly less expensive than commercial devices such as Rotorod® and Burkard Samplers that are used in the agricultural and health science industries. They are economical enough to be deployed in large numbers across broad landscapes. We evaluated their use for detecting airborne spread of ambrosia beetle frass that may contain infective spores of the fungi (*Ceratocystis lukuohia* and *C. huliohia*) that are responsible for Rapid `Ōhi`a Death (ROD), a newly documented pathosystem on Hawai`i Island. We compared performance of the new samplers to Rotorod® Model 20 Samplers by releasing synthetic polyethylene spheres (12–160 µm in diameter) and also *Xyleborus* spp. frass known to contain *C. lukuohia* and *C. huliohia* propagules under controlled laboratory and field conditions. Overall, the Active Environmental Sampler proved to be 3–4 times more effective in capturing polyethylene spheres and 2–3 times more effective in capturing frass than either the Passive or Rotorod® Samplers. Significant differences between the Passive and Rotorod® Samplers were not detected. For the frass release experiment, *C. lukuohia* DNA was detected once by qPCR in an Active Environmental Sampler and *C. huliohia* DNA was detected during two different trials, once with an Active Environmental Sampler and once with a Passive Environmental Sampler. No detections were made with Rotorod® Samplers. Both Active and Passive Samplers were used in the field for detection of airborne dispersal of *C. lukuohia* and *C. huliohia* at Orchidlands Estates in the Puna District of Hawai`i Island. We found that airborne dispersal of potentially infective beetle frass was uncommon over short distances with qPCR detections in up to 10% of weekly sampler collections.

INTRODUCTION

Rapid `Ōhi`a Death (ROD) is a vascular wilt disease of the endemic `ōhi`a lehua tree (*Metrosideros polymorpha*). This recently described pathosystem is caused by two new members of the fungal genus *Ceratocystis*, *C. lukuohia*, and *C. huliohia* (Barnes *et al.* 2018). ROD was first observed in 2010 in the Puna District of Hawai`i Island, identified as a significant pathogen in 2014 (Keith *et al*. 2015), and has since spread throughout Hawai`i Island. Long term integrity of native ecosystems in the Hawaiian Islands is threatened by the loss of this keystone native forest tree (Mortenson *et al.* 2016).

Precise mechanisms for how this disease spreads remain poorly known. Possible pathways include movement of contaminated wood, tree cutting tools, heavy equipment, movement of contaminated soil that contains infective propagules (Harrington 2013), and movement of ambrosia beetles (Coleoptera: Curculionidae subfamily Scolytinae) or other insect vectors carrying sticky ascospores (Wingfield *et al.* 2017). In addition, the air or waterborne spread of ambrosia beetle boring dust (hereafter referred to as frass) produced by native and introduced beetles that excavate galleries in the sapwood of injured, stressed, and dying trees is also a

potential means of spread (Iton 1961, Grosclaude *et al.* 1991, Luchi *et al.* 2013, Ocascio-Morales 2007, Souza *et al.* 2013, Roy *et al.* 2018). The movement of ambrosia beetle frass is of particular interest because it may play a central role in the epidemiology of the disease. Simple movement of infectious frass by feral ungulates, human foot traffic, and runoff from heavy rainstorms may be possible (Harrington 2013) and airborne transmission of some *Ceratocystis* spp. through contamination of wounds of healthy trees has been hypothesized (Iton 1961, Englebrecht *et al.* 2007, Harrington 2013).

Early outbreaks of this disease in the southeastern Puna District of Hawai`i Island and outlying areas to the west and northwest suggested that new cases were appearing in a pattern that was consistent with spread by the prevailing trade winds in the Wailuku River watershed west of Hilo, and the Ka`u and South Kona Districts west and southwest of Puna (Thomas Harrington, Iowa State University, personal observations). Although the airborne movement of other *Ceratocystis spp.* has been suspected (Harrington 2013) and demonstrated for *C. platani* by dispersal of sawdust associated with tree cutting (Luchi *et al.* 2013), there is little direct evidence that significant spread of these pathogens is mediated by this mechanism.

Most air samplers are designed for use in agricultural or urban settings rather than remote forest habitats and are susceptible to heavy rainfall and adverse weather conditions common in tropical climates. We designed two new air samplers for monitoring airborne particulates (windblown frass) to help understand dispersal pathways for the *C. lukuohia* and *C. huliohia* – one that is wind-driven and passive and one that is dependent on a power source. Unlike other commercial sampling equipment, these devices require minimal maintenance and are relatively inexpensive. Samplers were modeled on the traditional Hirst Spore Sampler (Hirst 1952) that relies on the use of sticky tape to collect airborne particulates. We compared performance of these new samplers to a commonly used commercial sampling device (Rotorod® Model 20) in controlled lab and controlled field cage experiments. Additionally, we demonstrated the relative efficacy of Active and Passive Samplers located in the field to detect the airborne dispersal of *C. lukuohia* and *C. huliohia* on Hawai`i Island.

METHODS

Sampler Design

Active Environmental Sampler

Active Environmental Samplers are based on minor modifications of the CDC (Centers for Disease Control) Gravid Mosquito Trap (Model 1712, John W. Hock Company, Gainesville, FL, $97/unit). When used for mosquito collecting, the trap is mounted vertically over a pan of fermenting water and a small 6-volt motor in a 3-inch diameter ABS pipe sucks ovipositing mosquitoes from the surface of the water and blows them into a mesh sample bag that covers the upper end of the pipe. When modified to collect airborne particulates, the 6-volt fan motor is replaced with a 12-volt fan motor (Catalog no. 238473, 12-volt DC electric motor, 170 mA, 4,840 rpm, Mabuchi Motor Company, Jameco Electronics, Belmont, CA, $4/unit) to increase air

flow and allow use with a 12-volt, 35 amp-hr gel cell battery (Interstate Batteries, catalog no. DCM0035, $80/unit). Total cost of the Active Sampler is < $200/unit. The sampler is mounted horizontally on a vertical section of ¾ inch electrical conduit with hose clamps, or attached to a tree trunk or branch with a bungee cord. A standard 1 inch X 3 inch microscope slide (Catalog no. 12-550-343, Fisherbrand frosted microscope slide, Fisher Scientific, Thermofisher Scientific, Waltham, MA) is coated with a thin layer of silicone grease (Catalog no. 335148, vacuum grease, Beckman Coulter, Brea, CA) with a cotton swab, covered with a strip of cellophane tape (Scotch® 810 Magic™ Tape) so that the writing block on the slide remains exposed, and then coated again with a second layer of grease. The first coat of grease makes removal of the cellophane tape easier for DNA extraction, while the second coat creates a sticky surface that captures fine particulates that are pulled over the surface of the slide by the 12-volt fan. Location and date information can be written on the exposed writing block. The slide is then placed at a 45° angle inside the cutout end of the ABS pipe (Figure 1) and held in place by friction so that airflow produced by the fan passes over the greased side of the slide and out the back of the sampler. By mounting the sampler horizontally rather than vertically, the ABS pipe provides the greased slide some protection from rain.

The sampler can be powered with a 12-volt, 35 amp-hr battery for up to seven days without being recharged. Battery life can be extended by connecting a small 10 watt, 12-volt solar panel (Catalog no. SLND 00542, Solarland, Ontario, CA) to the battery and mounting it on the electrical conduit beneath the sampler with a panel mount (Catalog no. COLO 00688, Solarland, Ontario, CA). Since airflow generated by the fan is constant, total volume of air passing over the slide can be quantified for any desired period of time by measuring air flow through the sampler (Figure 2).

Passive Environmental Sampler

Passive Environmental Samplers were designed to hold up to four greased microscope slides. Slides are supported in a small wooden box that holds the greased slides at a 45° angle. The box fits into a short section of 8-inch diameter galvanized ductwork that is attached to a sheet metal reducer (8–7 inches) (Figure 3). The ductwork assembly is supported by a 5/16-inch threaded rod that passes top to bottom through the sheet metal reducer. To allow the sampler to spin freely in the wind stream, the threaded rod passes through two 3/8-inch T-Nuts that are held in place by 5/16-inch nuts with a nylon washer (Figure 4). A sheet metal fin attached to the sheet metal reducer at the back of the assembly and secured in place with aluminum flashing allows it to rotate and face into the prevailing wind, eliminating the need for an external power source. Two versions of the sampler can be constructed, depending on prevailing wind speeds. For light winds, the sampler can be supported on a 5/16-inch threaded rod and made with an unreinforced sheet metal fin to make it more responsive to minor changes in wind direction and velocity. For windy locations, the sampler can be supported on a 3/8-inch threaded rod with ½-inch T-Nuts, and made with a fin that is reinforced with a strip of heavier gauge metal to reduce flexing and metal fatigue. Since Passive Environmental Samplers are non-quantitative and rely entirely on prevailing winds for airflow across the greased slides, they are most useful for detecting presence/absence of frass containing *Ceratocystis* over any

given sampling period. Passive samplers can be constructed from materials available in local hardware stores for < $50/unit. A full list of materials and description of how the sampler is assembled is available on request.

Figure 1. Active Environmental Sampler. The Active Environmental Sampler is a modified CDC (Centers for Disease Control) Gravid Mosquito Trap that contains a single greased slide at the cut-out end of the sampler (arrow). The slide is mounted at a 45° angle and held in place by friction with the sides of the tube. A 12-volt fan behind the slide pulls a steady stream of air over the sticky, greased surface.

Figure 2. Quantification of air flow across the surface of a greased slide in an Active Environmental Sampler. Airflow through the sampler is measured with a hand-held anemometer. Volume of air exposed to the surface of the greased slide (S) is calculated as height (h) of the slide X slide width X airflow distance (d) in one sec. Total volume sampled in one hour was calculated by multiplying by 60 to obtain volume/min and multiplying again by 60 to obtain volume/hr. Volume of air sampled by the Rotorod® was calculated as described by Frenz and Elander (1996) and Frenz et al. (1996) where Volume = (rod area) X (path diameter) X (motor speed) X π X (time sampled).

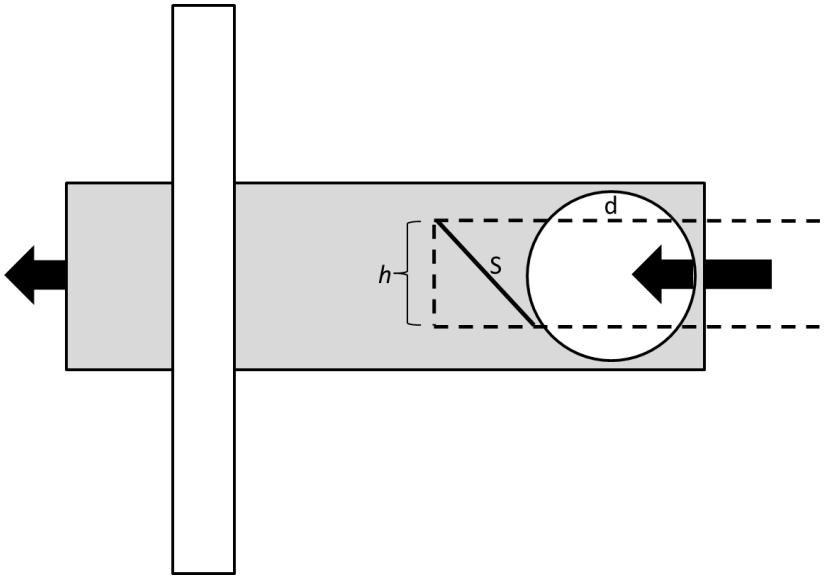
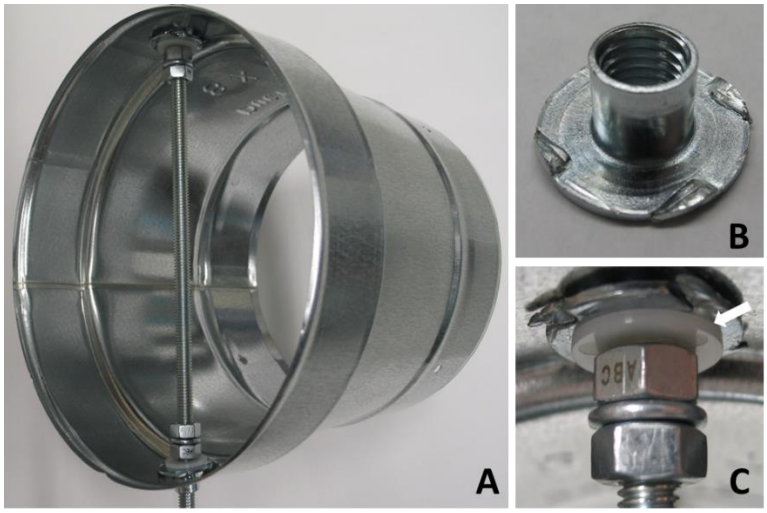


Figure 3. Passive Environmental Sampler. The sheet metal sampler (A) holds a wooden box (B) with four greased microscope slides that are mounted at a 45° angle. The sampler is placed at the end of a 10 ft. section of ¾-inch electrical conduit so that it can rotate freely in the prevailing wind (A). As wind passes across the surface of the greased slides, fine particulate matter is captured on their sticky, greased surfaces.

Figure 4. The Passive Environmental Sampler is constructed around an 8 inch to 7-inch sheet metal reducer (A) that is supported by a 5/16-inch threaded rod that passes top to bottom through the 8-inch diameter end of the reducer. To allow the sampler to spin freely, the threaded rod passes through two 3/8-inch T-Nuts (B) that are held in place by two 5/16-inch nuts with a lock washer (C). A nylon washer (arrow) is placed between the 5/16-inch nuts and the T-Nut to reduce friction.



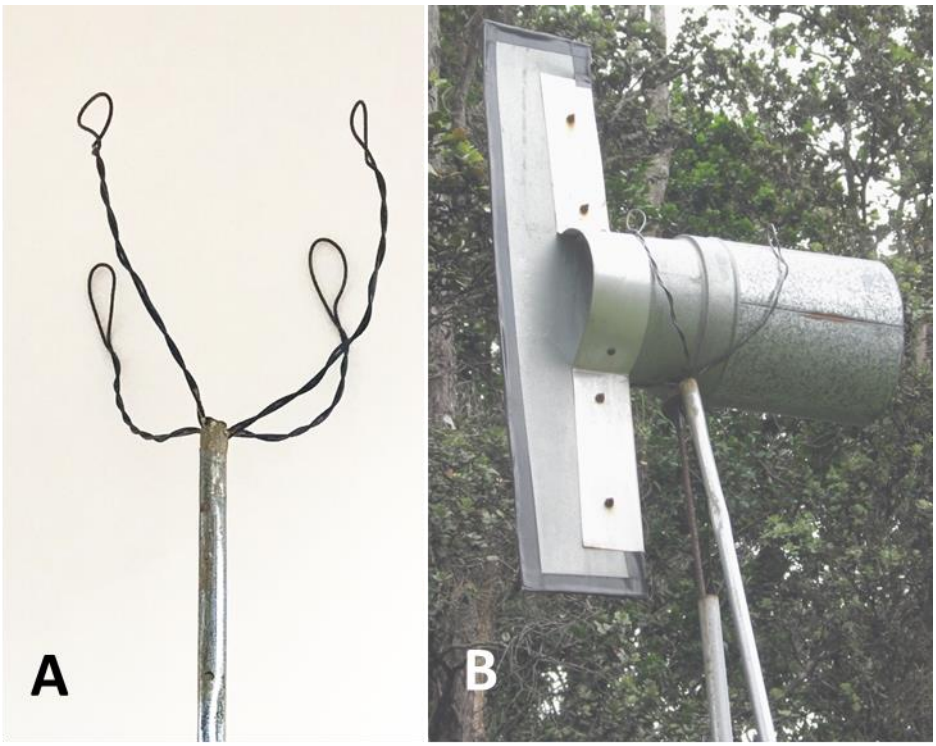
Sampler Deployment

Passive Environmental Samplers can be deployed on vertical 10 ft. sections of ¾-inch metal electrical conduit. The threaded rod that supports the Passive Environmental Sampler fits into the top of the section of conduit and rests on a 5/16- or 3/8-inch nylon washer that is supported by a 1½-inch fender washer at the top of the conduit (Figure 3). The sampler is then free to spin full 360° and face into the prevailing wind. By making a simple modification in sampler design to place the threaded rod in the center of the sampler and the threaded end of a 5/16-inch turnbuckle at each end, the sampler can be suspended at different heights above ground in the forest canopy (Figure 5). Active Environmental Samplers, by contrast, can be attached at any height on the conduit with hose clamps or on tree branches or trunks with an elastic bungee cord.

Electrical conduit can be supported by guy lines and stakes or can be attached to a metal or wooden fence post with heavy duty plastic zip ties or metal clamps designed for electrical conduit. Passive Environmental Samplers can be lifted into position or removed from the top of the electrical conduit with a "sampler picker" made from a 5 ft. section of metal conduit, with four, 18-inch lengths of twisted, 14-gauge galvanized fence wire that are glued into the end of the conduit with construction adhesive and shaped to form an open basket that can support the sampler as it is lifted into position (Figure 6).

Figure 5. Passive Environmental Sampler modified for deployment in the forest canopy. Note that the sampler has been moved to the center of the threaded rod. Turnbuckles added to the ends of the rod allow multiple samplers to be hung on the same line at different heights in the forest canopy.

Figure 6. Passive Environmental Sampler "Picker". The picker has four, 18-inch lengths of twisted 14-gauge galvanized fence wire that are glued into the end of a section of conduit with construction adhesive and shaped to form an open basket that can support the sampler as it is lifted on or off of a supporting pole.

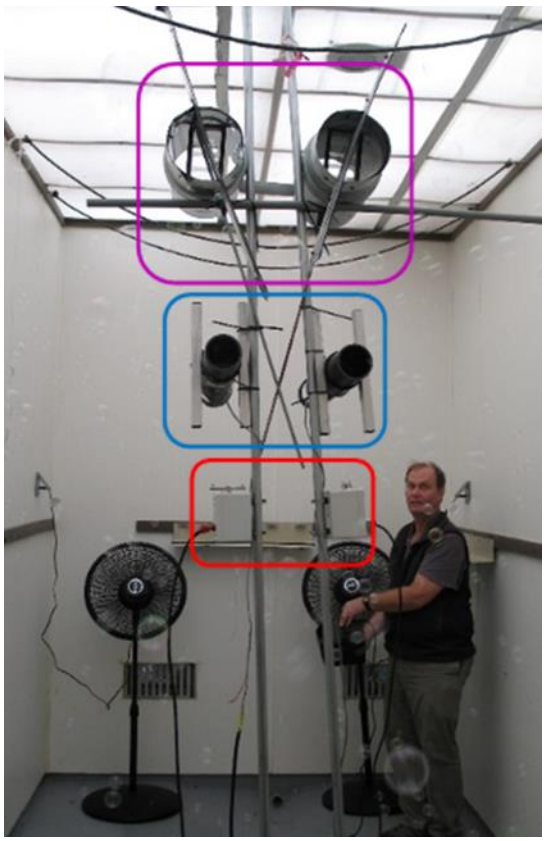


Sampler Evaluation Under Controlled Laboratory Conditions

Sampling efficiency of Passive and Active Environmental Samplers was compared to a commercial sampling device (Rotorod® Model 20) to determine number and range of particle sizes that each sampler can detect under relatively controlled conditions. Two 24-inch diameter electric fans were placed on either side of the back of a 5.5 x 2.3 x 3 m room and oriented so that wind flow was directed at a 45° angle against the rear wall. This allowed a circular circulation pattern to form in the room with air currents traveling to the rear along the ceiling and returning to the fans along the lower half of the room. Combined wind speed in the center of the room measured approximately 1 m/second with a hand-held anemometer. Since air currents differed slightly in speed and direction at different heights in the room, two, 2.4 m tall masts constructed of ½-inch metal electrical conduit and supported horizontally by one section of ½ inch PVC (polyvinyl chloride) conduit were placed in the center of the room. Masts were freestanding and supported by placing the ends into a ¾-inch PVC pipe that was immobilized

with cement in 10 X 10 X 12-inch cinder blocks. Paired samplers (two Passive, two Active, and two Rotorod® Model 20) were attached to the conduit adjacent to one another and rotated between the three vertical positions (140, 180, and 220 cm) above the floor for each trial (Figure 7). The paired Active Environmental and Rotorod® Samplers were powered by two 35 amp/hr gel cell batteries (one Active Environmental and one Rotorod® Model 20 per battery). Active Samplers held one greased microscope slide that was prepared as described earlier with Scotch® tape and silicone grease. Passive Environmental Samplers each held four greased microscope slides. The Rotorod® Model 20 Sampler held two square lucite rods, 20 mm in length and 5 mm in diameter that were smeared with silicone grease. All slides and rods were prepared inside a positive pressure PCR workstation (Airclean 600, Airclean Systems, Creedmoor, North Carolina) to reduce chances of extraneous contamination.

Figure 7. Release of microspheres under controlled conditions in a closed room with circulating airflow. Paired samplers were rotated between three vertical positions in a series of trials. Paired Rotorods® (red box, bottom), Active Environmental Samplers (blue box middle), and Passive Environmental Samplers (purple box top) were rotated between three vertical positions (140, 180, 220 cm from floor) in a series of trials. Airflow was adjusted by releasing soap bubbles into the room.



A 100 mg mix of polyethylene microspheres (Catalog Number CPMS-0.96, Cospheric LLC, Santa Barbara, California) ranging in size from 12–160 µm diameter (Mean = 80.0 $\pm$ 28.2 µm, Median = 73.8 µm, Mode = 60.9 µm) was carefully poured into a disposable 10 ml plastic serological pipette (Catalog no. 13-678-14A, Fisher Scientific, Thermofisher Scientific, Ipswich, MA) that was modified into a 10 mm diameter plastic pipe by removing the tapered tip from one end and the cotton plug from the other. Microspheres were blown from the pipette into the rear of the room with compressed air and allowed to circulate for each 4-hour trial. After each trial, slides were removed from Active and Passive Environmental Samplers and stored in a closed slide box until examined. Metal rod holders were removed from the Rotorod® Samplers and stored in plastic bags until rods could be removed with tweezers and examined on a microscope slide. Paired samplers were rotated to a new vertical position, microscope slides and rods were replaced, and trials were repeated until each pair of samplers was operated in each of the three vertical positions at least twice, for a total of eight trials.

Slides and lucite rods were examined with a 10X objective through an Olympus BH50 compound microscope (Olympus Life Sciences, Tokyo, Japan). Total number of captured beads and diameter of up to 50 beads was measured on each slide or lucite rod with CellSens software version 1.9 (Olympus Life Sciences, Tokyo, Japan). The flat surface of each square lucite rod was examined, even though most particles were collected on the two leading sides of the rod. Particle counts were square root transformed to normalize data and reduce skewness. Variance in particle counts for each sampler type was compared with a Levene's Test for equality of variance. Differences in particle counts between each of the three sampler types was compared by one-way ANOVA. To determine if sampler types collected different sized particles, differences in variance were compared with a Levene's Test for equality of variance and mean particle diameters for each sampler type and a random sample of 1,000 microspheres from the starting material were compared by one-way ANOVA.

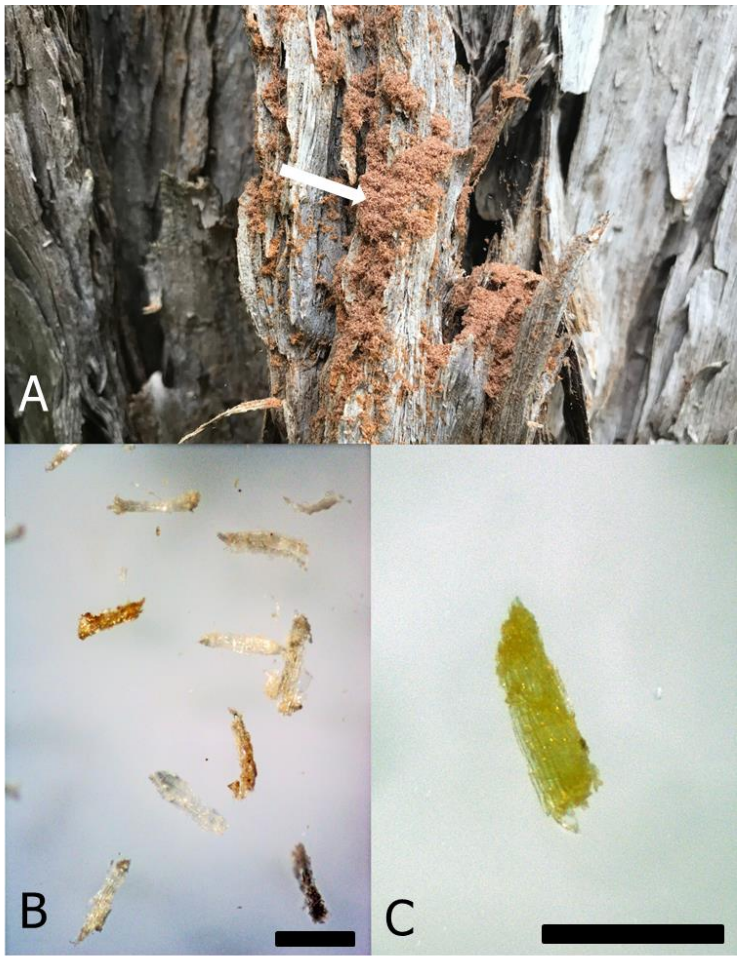
Sampler Evaluation Under Controlled Field Conditions

To compare samplers under controlled field conditions, *Xyleborus* spp. frass was collected from Waipunalei Ahupua`a near the town of Laupahoehoe and Waiakea Forest Reserve on Hawai`i Island from January–February 2018. About 1.5 g of frass was collected into each of 14, 15 ml Falcon tubes (Catalog no. 05-527-90, Fisher Scientific, Thermofisher Scientific, Waltham, MA) from the lower trunk of symptomatic trees (Figure 8). To insure homogeneity, each tube of frass was transferred to individual Whirl-Pak bags (Catalog no. 01-812-6C, Fisher Scientific, Thermofisher Scientific, Waltham, MA), mixed thoroughly, and returned to their respective collection tubes. To test for *Ceratocystis* infection, 15 mg of frass from each Falcon tube was transferred to 2-ml sterile screw cap tubes (Catalog no. 02-681-375, Fisher Scientific, Thermofisher Scientific, Waltham, MA) containing six, 3-mm zirconium beads (Catalog no. BAWZ 3000-300-23, OPS Diagnostics, Lebanon, NJ). Samples were extracted with NucleoSpin Plant II DNA extraction kit (Catalog no. 740770.250, Macherey-Nagel, Bethlehem, PA) following the manufacturer's protocol. Briefly, frass samples were disrupted with 300 µl of PL2 buffer and 10 µl of RNAse A (10 µg/µl) in 2.0 mL tubes with zirconium beads with a FastPrep 5G

homogenizer (MP Biomedicals, Santa Ana, CA) for two, 60 sec intervals at 6.5 m/s. The samples were heated at 65°C for 10 minutes between the two homogenization steps. Other extraction steps followed manufacturer recommendations for the NucleoSpin Plant II kit. DNA was eluted with elution buffer provided in the kit in a final volume of 100 µl. Screening for *C. lukuohia* and *C. huliohia* by qPCR followed methodology described by Heller and Keith (2018).

Eleven of 14 batches of frass were then soaked in 5 ml of 70% isopropyl alcohol for 30 minutes to kill *Ceratocystis* spp. propagules and then oven dried at 70°C for 48 hours. All frass samples were screened by qPCR for *C. lukuohia* and *C. huliohia* DNA (Heller and Keith 2018) after treatment with alcohol. About 30 mg of frass from each tube of treated and untreated frass was also carrot baited to check for fungal viability with a single carrot bait per tube (Moller and DeVay 1968).

Figure 8. A. Frass (arrow) produced by ambrosia beetles during excavation of natal galleries in a tree infected with *Ceratocystis*. The frass is frequently found on and under bark near the entrance of galleries. B and C. Frass particles produced by *Xyleborus* spp. are rectangular or cylindrical in form and measure approximately 500 µm in length. Bar = 500 µm.

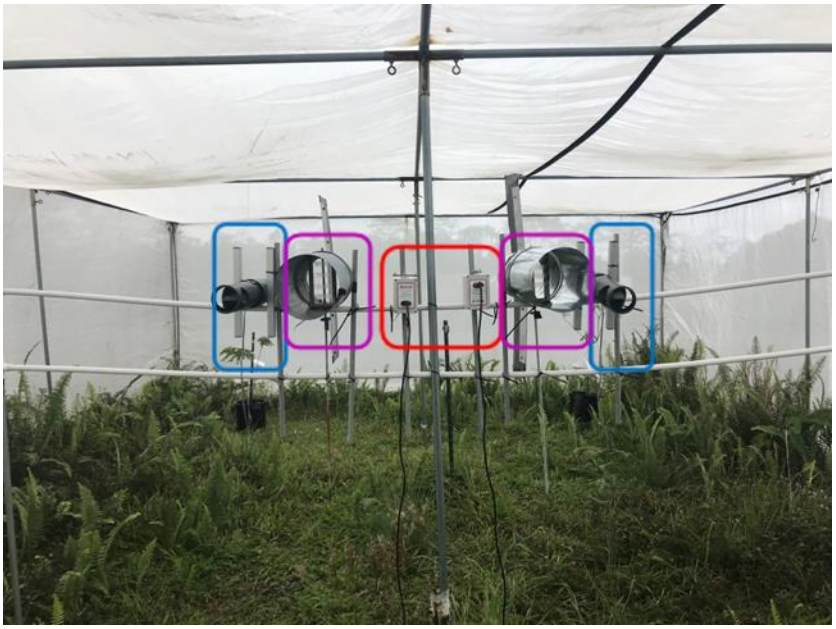


Release of beetle frass under controlled field conditions was done at facilities provided by U.S. Department of Agriculture, Agricultural Research Service, Daniel K. Inoye Pacific Basin Agricultural Research Center (USDA-ARS-DKI-PBARC) in Hilo. The experiment was conducted in a 6 x 3 x 15 m screened (1 mm mesh) outdoor cage with two "Heat Buster" fans (Model QBX4223, Triangle Engineering, 6.1 amps ½ HP) placed at one end of the cage to create a constant air flow from one end of the cage to the other. Samplers were set up so that two replicates for each sampler were run in each trial. Horizontal PVC piping was connected to three vertical support poles seven meters from the fans and 1.5 m from the ground. Samplers were attached 0.5 m apart on the horizontal PVC pipe and arranged so that those on the left side of the cage were mirror images of those on the right side of the cage, e.g. Rotorod®, Active, Passive on left and Passive, Active, Rotorod® on right (Figure 9). Samplers were systematically moved between each trial so that samplers were operated in each location on the horizontal PVC pipe three times. Twelve 15-minute trials were carried out during a 6-hour period where the average wind speed was 1.2 m/s with a range of 0.7–2 m/s. For each trial, ~300 mg of pooled treated or untreated ambrosia beetle frass was shot into the wind column from behind the fans using an air compressor and a 10 ml serological pipette as described for laboratory trials. After each trial, slides and rods were collected, samplers were rotated, and new slides and rods were replaced. A total of nine trials were conducted using frass treated with isopropyl alcohol, for a total of three replicates at each sampler position. An additional three trials were conducted with viable frass to demonstrate that treatment with isopropyl alcohol did not affect physical properties of the frass. For the final three trials, samplers were operated in each position once. Slides and rotorods were examined under a dissecting microscope for frass. All particles were counted (Figures 8B, 7C) and then measured with a compound microscope with a 10X objective and CellSens software to determine size ranges for particles from each sampler type. Frass counts were log transformed to normalize counts and reduce skewness. Variance among mean counts for each sampler type was tested with Levene's Test for equality of variances to determine whether significant differences were present. Mean differences in counts were compared by one-way ANOVA. Finally, a two-sample test of variance was done to compare counts of treated frass (Trials 1–9) with untreated frass (Trials 10–12).

To determine whether samplers can collect potentially infective frass, tape strips on each slide were cut into six vertical strips with a sterile scalpel blade and transferred to 2 ml screw-cap tubes containing 0.3 g of 800 um zirconium beads (Catalog no. BLBZ 800-250-34, OPS Diagnostics, Lebanon, NJ) and 0.3 grams of 100 um silica beads (Catalog no. BLBG 100-200-11, OPS Diagnostics, Lebanon, NJ). Both rods from each Rotorod® Sampler were placed in tubes containing the bead mixture. Both tape strips and rods were extracted using a QIAamp DNA Investigator Kit (Catalog no. 56504, Qiagen Inc., Germantown, MD) according to manufacturer's protocol, with an initial homogenization step at 4.5 m/s for 40 seconds in a FastPrep 5G homogenizer (MP Biomedicals, Santa Ana, CA). All samples were tested by qPCR for *C. lukuohia* and *C. huliohia* as described by Heller and Keith (2018) with minor modifications. Samples were run in triplicate for each *Ceratocystis* spp. in 96-well plates on a CFX96 Real-Time System (Bio-Rad Laboratories, Inc, Hercules, California). Triplicate negative

controls (ultrapure H_2O) and positive controls (synthetic gBlock® oligonucleotide containing *Ceratocystis* target sequences, Integrated DNA Technologies, Coralville, Iowa) were run on each plate.

Figure 9. Evaluation of samplers under controlled field conditions. Paired Active Environmental Samplers (blue), Passive Environmental Samplers (purple), and RotoRod® (red) Samplers were placed horizontally within a large mesh enclosure. Two large "Heat Buster" fans were placed at one end of the enclosure to provide continuous airflow and ~300 mg of frass/boring dust was blown into the wind column from behind the fans using an air compressor and a 10 ml serological pipette.



Field Evaluation of Samplers

To evaluate sampler function under field conditions, we set up two Active Environmental Samplers and three Passive Environmental Samplers on a 3-acre wooded residential property at Orchidlands Estates Subdivision in the Puna District of Hawai`i Island. Rotorods® were not evaluated in this trial. The property had ongoing `ōhi`a mortality due to ROD that was being managed by the landowner by periodically cutting dead and dying trees. Samplers were operated for 15 weeks, from July 12th to October 25th, 2016 within a 50 X 50-meter area next to a private residence. Samplers were set up approximately 30 m apart in the lawn (Lawn), next to a shed (Shed) and next to the residential water catchment tank (Tank). Two Active Environmental Samplers were operated on the pole supporting the Lawn Passive Environmental Sampler and powered with a 12-volt automobile battery with an attached solar panel to extend

battery life so that the Lawn received a total of three samplers (two Active and one Passive). A single Passive Environmental Sampler was placed in the Shed and Lawn locations. Active Environmental Samplers contained one microscope slide (one replicate) each, while Passive Environmental Samplers contained four slides (four replicates) each. Slides were made as described earlier with Scotch® tape coated with silicone grease and collected and replaced weekly.

When collected, tape strips on each slide were cut into six equally sized vertical strips with a sterile scalpel blade. Two-thirds of the tape was moved to a 2-ml screw cap tube containing 0.3 g of 800 µm zirconium beads and 0.3 grams of 100 µm silica beads for DNA extraction. The remaining 1/3 was left on the slide for later examination under a dissecting microscope to identify and count frass particles. Samples were homogenized and DNA was extracted and tested by qPCR as described earlier. Since low DNA copy numbers in extracts can reduce probability of detecting target sequences in qPCR reactions, we concentrated all extracted samples by ethanol precipitation and resuspended DNA pellets in 20 µl of Tris-Ethylenediaminetetraacetic acid (TE) buffer to improve detection sensitivity. Briefly, 2.5 µL of a 20 mg/ml glycogen solution (Catalog no. B1563, New England Biolabs, Ipswich, MA) were added to extracted DNA, and brought to a volume of 100 µl with TE buffer. Sodium acetate (3 M, pH 5.2) was added to increase the total volume to 116.7 µl. DNA was precipitated by adding three volumes (350 µl) of absolute molecular grade ethanol, followed by thorough mixing and incubation at -70°C for four hours. Precipitated DNA was pelleted by centrifugation at 20,000 g for 20 min at 4ºC. The supernatant was carefully removed and the pellet was carefully washed with 300 µl of 70% ethanol that was pre-chilled to -70ºC. The mixture was centrifuged at 20,000 g for 5 min at 4ºC. After the supernatant was carefully removed, the pellet was dried for 30 min in a 37ºC incubator with tube lids open to allow evaporation of alcohol and water. After air drying, the pellet was resuspended in 20 µl of TE buffer. All samples were tested again for *C. lukuohia* and *C. huliohia* as described by Heller and Keith (2018) after ethanol precipitation. Samples were considered positive if at least one of three replicates detected either *C. lukuohia* or *C. huliohia* by qPCR

Sampler Detection Limits

To determine probability of detecting different numbers of *Ceratocystis* spores on greased slides used in the Passive and Active Environmental Samplers, slides were spiked with known numbers of endoconidia from a laboratory culture of *C. lukuohia*. The fungus was cultured on Yeast Malt Agar (Catalog no. Y3127-500G, Sigma Chemical, St. Louis, MO) in a 150 mm plastic petri dish at room temperature until the fungus was confluent. The dish was then flooded with sterile distilled water and a bent glass rod was used to gently scrape the surface of the agar plate to release fungal endoconidia. The suspension of endoconidia was subsequently filtered through Miracloth (Catalog no. 475855-1R, EMD Millipore, Billerica, MA) to remove larger hyphal fragments. The final suspension of endoconidia was quantified with a hemocytometer. Twofold serial dilutions were prepared from the suspension at concentrations of 70, 35, 18, 9, 5, 3, and 1 spore per 10 µl of suspension. Droplets containing known numbers of endoconidia were spotted onto the surface of greased slides and dried in a 37ºC incubator for approximately 15

min. Eight replicate slides of each serial spore dilution (8 X 7 dilutions = 56 slides) were extracted using a QIAamp DNA Investigator Kit (Catalog no. 56504, Qiagen Inc., Germantown, MD). Tape was peeled from each slide as described previously, and transferred to a 2 ml vial containing a mixture of 0.3 mg of 100 µm glass beads and 0.3 mg of 800 µm zirconium beads. The vials were homogenized with a Fastprep 5G bead beater for 40 sec at 6.0 m/sec with kit extraction buffers and processed according to kit instructions. Extracted DNA was eluted in 40 µl of elution buffer and tested by qPCR with either three or nine replicates/sample to determine effect of replicate number on ability to detect extremely low copy numbers of the *Ceratocystis* cerato-plantanin gene (Heller and Keith 2018). Results were analyzed by probit regression after data were log transformed to control for variability in variance at each serial dilution (MedCalc Statistical Software version 18.2.1, MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018).

Statistical Analysis

Statistical analyses were performed with either Microsoft Excel (2016), SYSTAT (2011) or R version 3.4.0 (R Core Team 2017).

Metadata

Data from this study are archived as comma-separated value (csv) files. Metadata was created to describe each file using MetadataWizard, version: 2.158.0 (ScienceBase-Catalog, U.S. Geological Survey, https://www.sciencebase.gov/catalog/).

RESULTS

Volume of Air Sampled by Active and Rotorod® Samplers

The calculated sampling rate (volume/hour) for the Active Environmental Sampler was 15.43 m^3/hr. By contrast, calculated sampling rate for the Rotorod® Sampler was 0.86 m^3/hr.

Comparison of Different Samplers under Controlled Conditions

Controlled Laboratory Experiment

Microsphere Counts

During the course of the controlled laboratory experiment, paired Active, Passive and Rotorod® Samplers (six total) were rotated eight times between the three vertical positions in the room. Of the 48 individual sampler exposures that were obtained, only 44 could be used for analysis because of unexpected problems with battery connections. Particle counts were pooled by sampler type for analysis since samplers received roughly equal exposure in the three positions, and plots of mean particle counts at each vertical position did not differ significantly within individual trials (data not shown).

Microsphere counts from all samplers ranged from 0–1,366 particles per sampler (Table 1) with a mean 579 ± 93.53 for Active Samplers, 158 ± 41.54 for Passive Samplers, and 133 ± 17.80 particles for Rotorods®. Active Environmental Samplers collected significantly more microspheres than either Passive Environmental or Rotorod® Samplers ($P < 0.001$, $F = 31.974$,

df = 2), but also had the highest variance (Levene's Test F = 5.918, P = 0.006). While microsphere counts did not differ significantly for either Passive Environmental or Rotorod® Samplers (P = 0.988) (Table 1, Figure 10), variance for Rotorod® Samplers was lowest (Table 1, Figure 10).

Table 1. Particle counts for Active Environmental Sampler, Passive Environmental Sampler, and Rotorod® Samplers when exposed to microspheres under controlled conditions in a closed room with circulating airflow.

	Active	Passive	Rotorod®
Replicates	16	16	14
Range	0–1,366	2–677	4–210
Median	536	118	159
Mean	579	158	133
Std. Error	93.529	41.541	17.797
Standard Dev	374.115	166.165	66.59
Variance	139,961.8	27,610.86	4,434.247

Microsphere Size

Microspheres that were released into the closed room with circulating airflow ranged from 12.8–160 µm in size with a mean diameter of 80.001 $\pm$ 28.225 µm (N = 1,000, Table 2, Figure 11). Active Environmental Samplers collected particles (Mean = 84.154 $\pm$ 22.736 µm) that were significantly larger in mean diameter to microspheres in the pre-release material (P < 0.001, F = 193.03, df = 3). Passive Environmental Samplers collected particles (Mean = 82.12 $\pm$ 26.678 µm) that did not differ significantly in diameter from either the starting material (P = 0.193) or particles collected by Active Environmental Samplers (P = 0.230). Rotorods®, by contrast, collected particles that were significantly smaller in diameter (Mean = 65.515 $\pm$ 18.905 µm) than those in starting material or those collected by Active and Passive Environmental Samplers (P < 0.001). Variance of particle diameters differed significantly among the three sampler types, with lowest variance in diameter for particles collected by Rotorods® (Levene's Test, F = 58.071, P < 0.0001).

Figure 10. Box Plot of microsphere counts from Active Environmental, Passive Environmental and Rotorod® Samplers. Active Environmental Samplers collected significantly more particles than either Passive Environmental or Rotorod® Samplers, but also had the widest variance of the three methods. Microsphere counts did not differ significantly for either Passive or Rotorod® Samplers. Letters above each graph indicate statistical differences, e.g. AB indicates that the treatment did not differ significantly from either A or B. The central horizontal line marks the median of each sample, the length of each box shows the range within which the central 50% of the values fall (between 25th and 75th percentiles), and the vertical blue lines and markers designate the 10th and 90th percentiles. Values between the inner and outer fences are plotted with asterisks. Values beyond the outer fences are plotted with empty circles.

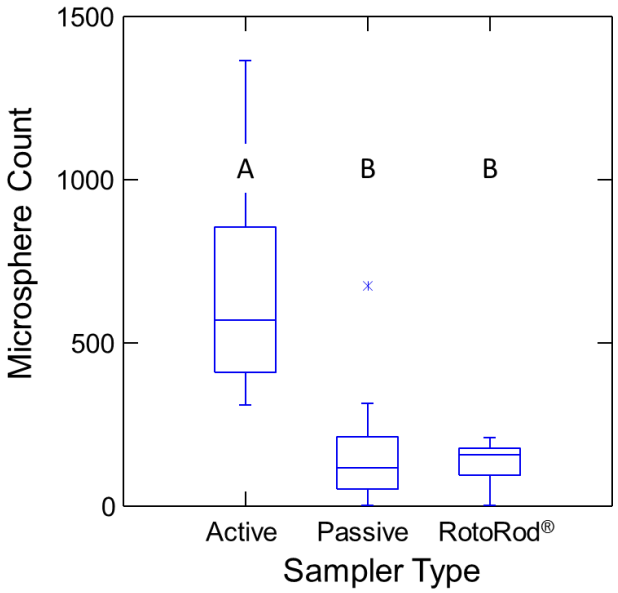
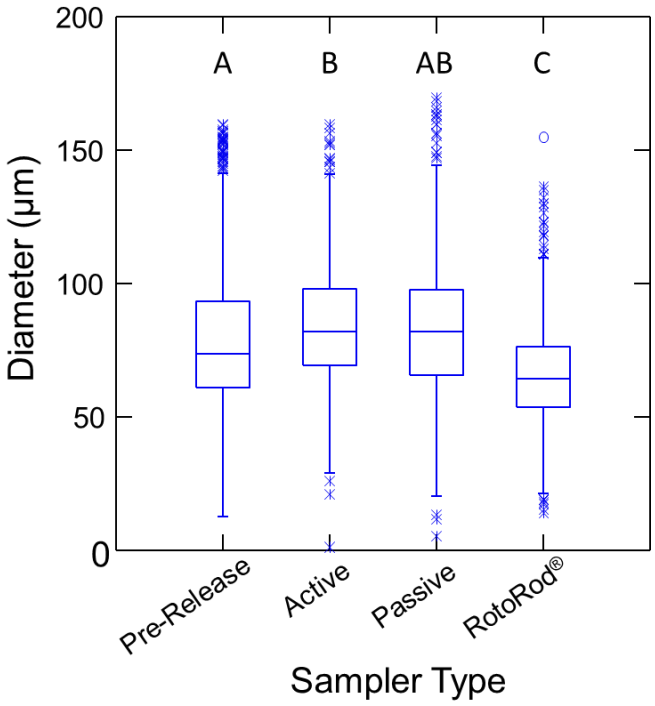


Table 2. Microsphere diameters (µm) that were measured prior to release (Pre-Release) and after capture by Active Environmental, Passive Environmental, and Rotorod® Samplers under controlled conditions in a closed room with circulating airflow. Particles collected by Rotorods® were significantly smaller in diameter and had a slightly smaller size range and lower variance in size than those collected by the other samplers (Levene's Test, $F = 58.071$, $P < 0.0001$).

	Pre-Release	Active	Passive	Rotorod®
N	1,000	976	942	1,856
Range	12.8–160	1.5–160	5.7–170	14.2–155
Median	73.82	82.159	82.159	64.452
Mean	80.001	84.154	82.12	65.515
Std. Error	0.893	0.728	0.869	0.439
Standard Dev	28.225	22.736	26.678	18.905
Variance	796.63	516.936	711.723	357.416

Figure 11. Box Plot of microsphere diameters from Active, Passive and Rotorod® Samplers. Active Environmental Samplers collected particles that were significantly larger than those in pre-release starting material. Rotorods® by contrast collected particles that were significantly smaller than those in starting material and those on Active and Passive Environmental Samplers. Letters above each graph indicate statistical differences, e.g. AB indicates that the treatment did not differ significantly from either A or B. The central horizontal line marks the median of each sample, the length of each box shows the range within which the central 50% of the values fall (between 25th and 75th percentiles), and the vertical blue lines and markers designate the 10th and 90th percentiles. Values between the inner and outer fences are plotted with asterisks. Values beyond the outer fences are plotted with empty circles.



Controlled Field Experiment

We released both isopropyl alcohol treated and untreated *Xyleborus* frass into a large field cage to compare sampler types under controlled field conditions. Twelve trials were conducted, with sampler position changing at each trial to control for effects of sampler position in the air stream. The first nine trials used pooled alcohol-treated frass while the last three trials used untreated and potentially infective frass. A comparison of variance in overall particle counts for all three sampler types was not significantly different for trials 1–9 vs. trials 10–12 ($F = 1.367$, $df = 51, 16$, $P = 0.501$), so all particle count data was combined for analysis.

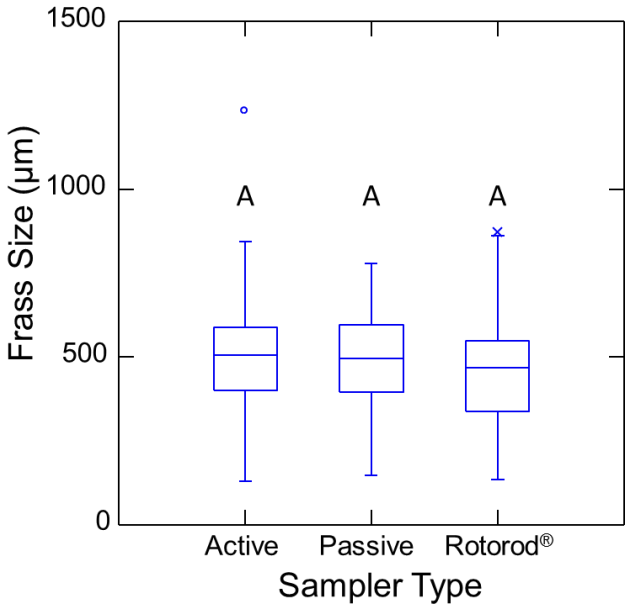
After treatment with isopropyl alcohol, seven samples tested positive for *C. lukuohia*, two tested positive for *C. huliohia*, and two were negative by qPCR. None of the carrot baited frass samples were viable for either *C. lukuohia* or *C. huliohia* after isopropyl alcohol treatment (n = 11). The final three batches of untreated frass tested positive for *C. huliohia* by qPCR and one batch was viable by carrot baiting.

Frass ranged in length from 128–887 µm with a mean of 485.534 ± 6.13 µm (N = 568). There was no significant difference in frass size among the three sampler types when data was normalized by square root transformation (F = 2.18, df = 2, P = 0.122) (Figure 12, Table 3).

Table 3. Measurements of frass collected with different sampler types. No significant differences in frass size were detected (P = 0.122).

	Active	Passive	Rotorod®
N	339	92	138
Range	128.9–1231.7	142.7–774.0	149.3–887.5
Median	503.2	491.5	481.4
Mean	494.3	483.7	465.3
Std. Error	7.8	15.1	12.9
Standard Dev	143.6	145.3	151.5
Variance	20,622.7	21,113.0	22,947.5

Figure 12. Box Plot of frass sizes collected on different sampler types. No significant differences in frass size were detected (P = 0.122). Letters above each graph indicate statistical differences, e.g. AB indicates that the treatment did not differ significantly from either A or B. The central horizontal line marks the median of each sample, the length of each box shows the range within which the central 50% of the values fall (between 25th and 75th percentiles), and the vertical blue lines and markers designate the 10th and 90th percentiles. Values between the inner and outer fences are plotted with asterisks. Values beyond the outer fences are plotted with empty circles.

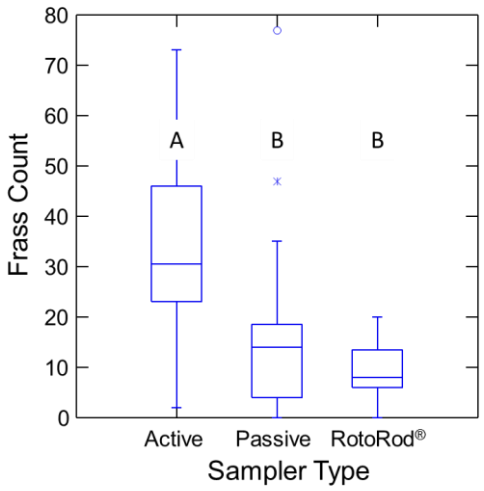


Mean frass counts for Active Environmental, Passive Environmental and Rotorod® Samplers were 33.52 ± 3.74, 16.26 ± 3.71, and 9.5 ± 1.11, respectively (Table 4). Active Environmental Samplers collected significantly more frass than either Passive Environmental Samplers (P=0.002) or Rotorod® Samplers (P < 0.001). Differences between Passive Environmental Samplers and Rotorod® Samplers were not significant (P = 0.354) (Table 4, Figure 9). Variance of particle counts differed significantly among the three samplers, with the lowest variance observed for Rotorod® Samplers (Levene's Test, F = 5.738, P = 0.005) (Table 4, Figure 13).

Table 4. Frass counts for Active Environmental, Passive Environmental, and Rotorod® Samplers.

	Active	Passive	Rotorod®
Replicates	25.000	23.000	24.000
Range	2–73	0–77	0–20
Median	30.000	13.000	8.000
Mean	33.520	16.261	9.500
Std. Error	3.743	3.712	1.105
Standard Dev	18.715	17.800	5.413
Variance	350.260	316.838	29.304

Figure 13. Box Plot of frass counts from Active Environmental, Passive Environmental, and Rotorod® Samplers. Active Environmental Samplers collected significantly more particles than either Passive Environmental or Rotorod® Samplers. Variance among particle counts for Rotorod® Samplers was significantly lower than either Active or Passive Environmental Samplers. Letters above each graph indicate statistical differences, e.g. AB indicates that the treatment did not differ significantly from either A or B. The central horizontal line marks the median of each sample, the length of each box shows the range within which the central 50% of the values fall (between 25th and 75th percentiles), and the vertical blue lines and markers designate the 10th and 90th percentiles. Values between the inner and outer fences are plotted with asterisks. Values beyond the outer fences are plotted with empty circles.



Ceratocystis lukuohia DNA was detected by qPCR once from a Passive Environmental Sampler with two frass particles (Table 5). *Ceratocystis huliohia* DNA was detected twice on slides from Active Environmental Samplers in different trials — once on a slide with 41 frass particles and

once from a slide containing 35 frass particles. There were no positive detections from Rotorod® Samplers (Table 5). Two detections occurred with treated frass and one detection occurred with untreated frass.

Table 5. *Ceratocystis lukuohia* and *huliohia* DNA detections from slides that were exposed in different sampler types after controlled release of frass. DNA was not detected on Rotorod® Samplers.

Trial Number	Trap Type	Detection	Frass Particle Count
8 — Treated	Active	*C. huliohia*	41
9 — Treated	Passive	*C. lukuohia*	2
10 — Untreated	Active	*C. huliohia*	35

Sampler Detection Limits

A probit analysis was used to determine confidence intervals for detecting serial dilutions of endoconidia that were spotted on tape strips, processed for DNA extraction, and then tested for *C. lukuohia* (Table 6) These were evaluated with qPCR tests that were run in replicates of three or nine. Based on probit analysis of qPCR tests (eight slides for each spore dilution), there was a 95% probability that 214 endoconidia (80–3,030) can be detected on material extracted from tape strips when three qPCR replicates are performed. Assay sensitivity increased to 53 endoconidia (25–307) when number of qPCR replicates was increased to nine (Table 7).

Table 6. Number of positive slide extractions for each spore dilution out of a total of eight that were positive for *C. lukuohia* when qPCR reactions were repeated either three or nine times. Note increased sensitivity as number of qPCR replicates are increased.

	Positive Slide Extractions	
# Spores	qPCR = 3	qPCR = 9
70	7	8
35	4	6
16	2	6
8	3	6
4	1	4
2	0	1
1	0	0

Table 7. Probability and 95% confidence intervals for detecting endoconidia of *C. lukuohia* based on either three or nine qPCR replicates. Increasing the number of qPCR replicates improved the probability of detecting fewer endoconidia.

qPCR Replicates = 3				qPCR Replicates = 9			
Probability	Spores	95% Confidence interval		Probability	Spores	95% Confidence interval	
0.01	1.1	0.1	2.9	0.01	0.3	0.0	0.9
0.02	1.5	0.1	3.7	0.02	0.4	0.0	1.1
0.025	1.7	0.2	4.1	0.025	0.5	0.1	1.3
0.05	2.6	0.4	5.6	0.05	0.8	0.1	1.7
0.1	4.3	1.0	8.1	0.1	1.2	0.3	2.4
0.2	7.7	2.8	13.4	0.2	2.1	0.7	3.8
0.25	9.6	4.1	16.8	0.25	2.7	1.0	4.6
0.5	23.7	13.6	54.4	0.5	6.4	3.5	11.1
0.75	58.4	30.5	260.3	0.75	15.2	9.0	37.9
0.8	73.0	36.4	393.7	0.8	18.9	10.9	53.5
0.9	131.3	56.4	1,195.1	0.9	33.3	17.3	137.5
0.95	213.5	80.0	3,030.1	0.95	53.3	24.8	307.0
0.975	325.2	107.8	6,829.2	0.975	80.0	33.6	622.1
0.98	368.7	117.6	8,703.9	0.98	90.3	36.7	768.4
0.99	530.7	151.6	1,7641.8	0.99	128.4	47.5	1,423.1

Field Evaluation of Passive and Active Samplers

A total of 75 weekly sampler exposures (5 samplers X 15 weeks) were collected during the study period, 30 from the paired Active Environmental Samplers and 45 from the three Passive Environmental Samplers. Of 207 individual slides that were collected from Active and Passive Samplers, 30 were from Active Environmental Samplers and 181 were from Passive Environmental Samplers.

Slides collected from the samplers were processed for extraction of DNA and then tested both before and after eluates were concentrated by ethanol precipitation. Before concentration, two slides collected from one Active and one Passive Environmental Sampler were positive for *C. lukuohia*. After ethanol precipitation and DNA concentration, the number of positive *C. lukuohia* DNA detections from slides increased to seven. The number of qPCR positive DNA detections of *C. huliohia* increased from none prior to DNA concentration to eight detections after concentration.

Ceratocystis lukuohia and/or *C. huliohia* were detected by qPCR in DNA extracts from a total of 10 tape strips or 4.8% (10/207) of all slides that were collected or 9.3% (7/75) of all weekly sampler exposures during the study (Table 8). *Ceratocystis lukuohia* was detected on tape strips collected from two Passive Environmental Samplers during the 15-week experiment, once at the Shed and once at the Lawn location. Active Environmental Samplers detected *C. lukuohia*

three times in the Lawn (Table 8). *Ceratocystis huliohia* was detected on tape strips from both Active and Passive Samplers at all three locations (Table 8). Both species of *Ceratocystis* were detected on tape strips from Active and Passive Environmental Samplers in the Lawn and the Passive Environmental Sampler at the Shed (Table 8).

Table 8. Slides that were positive for *C. lukuohia* and *C. huliohia* at Orchidlands Estates during sampler exposures between July 12 and October 18, 2016. Data are based on analysis of concentrated DNA from tape strip extractions. Cq values represent the qPCR cycle where fluorescence crossed a threshold value for being classified as positive. Replicates indicate number of replicates out of 3 that were positive for either *C. lukuohia* or *C. huliohia*.

Sampler	Week	Cq *lukuohia*	Replicates	Cq *huliohia*	Replicates
Lawn Active 1	1	38.66	1	-	-
Shed Passive (Slide 1)	3	34.81	3	37.48	1
Shed Passive (Slide 4)	3	-	-	36.88	2
Lawn Active 1	3	37.01	2	-	-
Lawn Passive (Slide 2)	5	38.40	1	37.66	1
Lawn Passive (Slide 3)	5	35.46	3	36.48	3
Shed Passive (Slide 4)	5	35.82	1	36.56	1
Tank Passive (Slide 2)	5	-	-	37.22	1
Tank Passive (Slide 4)	5	-	-	36.96	2
Lawn Active 2	7	37.49	1	36.17	1

When detection rates for Active and Passive Environmental Samplers were compared based on weekly sampler exposures, Active Environmental Samplers had a detection rate (10%, 3/30) that was similar to Passive Environmental Samplers (8.9%, 4/45) (Fisher Exact Test, P = 1.000). When detection rates were compared by slides, Active Environmental Samplers had a detection rate of 10% (3/30) while Passive Environmental Samplers had a detection rate of 3.9% (7/181), but this difference was not significant (Fisher Exact Test, P = 0.155).

All detections of *C. lukuohia* and *C. huliohia* occurred during the first seven weeks of the study (Table 9). Positive detections were not clearly associated with either tree felling by the property owner (P = 0.282, Fisher Exact Test) or two large tropical storms that caused high winds and excessive rainfall on Hawai`i Island. Ambrosia beetle frass was found on 10 of the 207 slides and wood chips/sawdust were found on 3/207 slides that were examined. Two of the ten slides that were positive for *C. lukuohia* and *C. huliohia* had visible frass, but there was no clear

association between positive detections and presence or absence of either frass (P = 0.560, Fisher Exact Test) or wood chips (P = 1.000, Fisher Exact Test) (Table 9).

Table 9. Airborne detections of *Ceratocystis lukuohia* and *C. huliohia* by qPCR assay, visual presence of beetle frass and wood chips on slides, management activities, and major storms during the Orchidlands Estates study in 2016. All detections occurred during the first seven weeks of the study but were not consistently associated with presence of beetle frass or wood chips on the slides, tree felling, or tropical storms.

Week	Sampling Start Date	Sampling End Date	Detections	Frass	Wood Chips	Tree Felling	Tropical Storms
1	7/12/2016	7/19/2016	*C. lukuohia*	Yes		No	
2	7/19/2016	7/26/2016			Yes	Yes	Darby
3	7/26/2016	8/2/2016	*C. lukuohia*, *C. huliohia*			Yes	
4	8/2/2016	8/9/2016				No	
5	8/9/2016	8/16/2016	*C. lukuohia*, *C. huliohia*	Yes	Yes	Yes	
6	8/16/2016	8/23/2016			Yes	No	
7	8/23/2016	8/30/2016	*C. lukuohia*, *C. huliohia*			Yes	
8	8/30/2016	9/6/2016				Yes	Madeline
9	9/6/2016	9/13/2016				Yes	
10	9/13/2016	9/20/2016		Yes		No	
11	9/20/2016	9/27/2016		Yes		No	
12	9/27/2016	10/4/2016				No	
13	10/4/2016	10/11/2016				Yes	
14	10/11/2016	10/18/2016		Yes		No	
15	10/18/2016	10/25/2016				No	

DISCUSSION

Sampler Comparison

A wide variety of either active or passive methods have been developed to sample airborne particulates such as fungi, bacteria and viruses (West and Kimber 2015, Mahaffee and Stoll, 2016). Active methods rely on a mechanical device to collect particulates through suction, impaction, or electrostatic charge, while passive methods are dependent on gravity, wind, rainfall, or inertia to bring particulates to a stationary sampler. Examples include filtration systems that pull air through a porous matrix (Aizenberg *et al.* 2000), electrostatic devices that

collect particulates based on charge (Schneider *et al.* 2007, Han *et al.* 2015), and impact-collection methods that either rely on rotating rods or vanes, e.g. Rotorod® (Di-Giovanni 1998, Eaton *et al.* 2017) or passage of air across a fixed surface (Hirst 1952, Carvalho et al. 2008). Other types rely on stationary ground samplers to collect airborne particulates as they settle or are carried to the ground in rainfall (Bittner *et al.* 2017, Chen *et al.* 2018), passive deposition of particulates on filter paper or media (Schweigkofler *et al.* 2004, Luchi *et al.* 2013), and a variety of simple home-made devices based on the capture of airborne particles on a sticky surface (von Qualen and Yang 2006, West and Kimber 2015). Variations on these methods have been used in combination with traditional microscopy and more recently molecular methods, image analysis, and optical sensing to identify and quantify airborne spores of a variety of plant pathogens (Rogers *et al.* 2008, Dedeurwaerder *et al.* 2011, Kaye *et al.* 2005, Lei *et al.* 2018).

Because of the high cost of most commercial spore samplers, the need to collect samples in multiple remote locations across large landscapes, and the high maintenance costs associated with operating the devices, we tested two new designs that are notably less expensive than the Rotorod® Model 20 (~$800/unit). The Active Environmental Sampler can be assembled from a CDC gravid trap, 12-v replacement motor, and 35 amp-hr, 12-volt gel cell battery for less than $200 while the Passive Environmental Sampler can be constructed easily from materials available in a local hardware store for less than $50. Both devices have relative advantages and disadvantages. The Active Environmental Sampler uses a small electric fan to draw air across a greased slide, is desirable for closed canopy forests with limited wind, and is semi-quantitative since air flow across the collecting surface can be calculated. However, cost is roughly 3–4 times higher than the Passive Environmental Sampler and routine maintenance in the field is needed to check on motor operation and battery charge. By contrast, the Passive Environmental Sampler is very inexpensive, can be deployed in large numbers across remote landscapes, and is easily modified for placement at different heights in the forest canopy. Maintenance costs are significantly lower because electrical power is not required for operation.

Passive and Active Environmental Samplers performed extremely well when tested side by side with the Rotorod® Model 20. Performance as measured by number of microsphere and frass particles that were captured over defined exposure conditions was significantly better for Active Environmental Samplers in both controlled lab and field experiments. The Active Environmental Sampler collected 3–4 times more microspheres than either Passive or Rotorod® Samplers (Table 1). While Rotorod's® favored capture of smaller sized particles of more uniform size than either Passive or Active Environmental Samplers (Table 2), median particle sizes captured by all three samplers were comparable, ranging from 66 µm for Rotorod® Samplers to 82 µm for Active and Passive Environmental Samplers (Table 2). Active Environmental Samplers were roughly twice as effective (33.52 ± 3.74 frass particles) than either Passive Environmental (16.26 ± 3.71 frass particles) or Rotorod® Samplers (9.5 ± 1.11 frass particles) in capturing frass.

Differences in sampler effectiveness may be related directly to collecting area and volume of air that is sampled. Based on calculations of surface area and volume of air, Active Environmental

Samplers (15.43 m^3/hr) are superior to Rotorod® Samplers (0.86 m^3/hr) in terms of cubic meters of air that can be sampled over any given period of time. Passive Environmental Samplers are comparable to or even superior to Rotorod® Samplers when ambient wind speeds are high enough to maintain constant air flow through the device because the exposed collecting area (950 mm^2 per slide, 3,800 mm^2 total) is more than 13 times larger than available surface area on the paired lucite rods in a Rotorod® (140 mm^2 per rod, 280 mm^2 total). Slides are also less likely than narrow lucite rods to become saturated with particulates during long exposures in the field and do not require special holders for examination by microscopy.

Our findings are in general agreement with previous studies that have compared Rotorod® Samplers with other devices for collecting airborne particulates (Crisp *et al.* 2013, Eversmeyer *et al.* 1975). Prior comparisons have detected few significant differences between sampler types (Eversmeyer *et al.* 1975, Heffer *et al.* 2005, Levetin *et al.* 2000) or specific differences that were related to sampling target or environmental conditions (Banks and Di Giovanni 1994, Crisp *et al.* 2013, Chen *et al.* 2018). Most have concluded that a variety of sampler types can be used for following trends in pollen or spore numbers over time. We found that all three samplers can be used effectively for monitoring airborne dispersal of *Ceratocystis.* In situations where large numbers of samplers need to be deployed across a large landscape in remote locations, Passive Environmental Samplers may be preferable to either Rotorod® or Active Environmental Samplers because of their low cost, simple maintenance, and high capacity for collecting particulates.

Airborne Detection of Ceratocystis

The atmosphere is a common pathway for movement of microorganisms, including fungi and their spores, bacteria, and viruses (Nunez *et al.* 2016). Some fungi have important adaptions for facilitating spread over long distances. Adaptations include thick, pigmented spore walls to provide protection against ultraviolet radiation and desiccation, fruiting bodies that facilitate release of spores into the air column, and life history strategies that promote release of enormous numbers of spores to increase the odds of reaching new hosts (Brown 1997). These adaptations reach their extremes among species of mildew and rust fungi that produce vast quantities of spores capable of being spread for thousands of kilometers on prevailing winds (Brown and Hovmøller 2002).

The importance of airborne dispersal for spread of *Ceratocystis* is unclear. Unlike mildew and rust fungi, species of *Ceratocystis* produce sticky ascospores on perithecia that are adapted for attachment to and movement by insects (Wingfield *et al.* 2017). Aleurioconidia that form deep within the vascular tissue of infected trees can be liberated in frass produced by burrowing ambrosia beetles and possibly carried from tree to tree on their surface or in gut contents. Frass that is expelled from boring tunnels may be picked up by wind, moved short distances by rain splash, or carried in flowing streams to new hosts (Harrington 2013). Evidence for airborne spread of *Ceratocystis* inoculum is mostly speculative (Iton 1959, 1961, Luchi *et al.* 2013), however, and it is unclear whether it plays a significant role in spread of the infection. While beetle frass and sawdust associated with tree felling has been detected in air samples near

infected trees, there is no definitive evidence that airborne inoculum can actually lead to new infections.

We successfully detected beetle frass and wood chips by visual inspection of slides collected from Active and Passive Environmental Samplers at Orchidlands Estates. These observations were similar to those reported by Iton (1959, 1961) who detected airborne beetle frass on greased slides that were placed near cacao trees infected with *C. cacaofunesta*. We also amplified both *C. lukuohia* and *C. huliohia* by qPCR from DNA that was extracted from tape strips on the slides. Detections were uncommon (10% of weekly collections from Active Environmental Samplers and 8.9% of weekly collections from Passive Environmental Samplers) in spite of their close proximity (< 50 m) to infected trees with abundant frass. We attempted to relate detections on the study site with management actions by the land owner and passage of two tropical storms, but the limited duration of the study and low number of positive detections precluded a robust statistical analysis. Three of four weekly detections were made when trees were cut, suggesting a possible correlation between tree cutting and positive detections as described by Luchi *et al.* (2013), but these were not significantly different from weeks where cutting did not take place. We also failed to find significant associations between positive detections and presence of wood chips and frass (Table 8). It is likely that factors other than tree felling, including rainfall, beetle phenology, distance to felled trees, and wind speed and direction may have influenced detection. No detections were made during passage of tropical storms Darby and Madeline, suggesting that the combination of heavy rainfall with wind may have washed frass out of the atmosphere or made it too wet to become airborne. A more robust, replicated study design of longer duration that includes unmanaged control sites may help to clarify whether there is an association between specific environmental factors, management activities, and airborne detections.

Other factors that may have influenced detections include sensitivity of the qPCR assay used for amplifying *Ceratocystis* DNA, ability to isolate and purify DNA from contaminants that can inhibit PCR reactions, relative extraction efficiency from slides that may contain limited amounts of DNA and number of qPCR replicates and stochastic probability of detecting low concentration template DNA. We found that ethanol precipitation and concentration of DNA template from initial extractions led to a substantial improvement in detecting *Ceratocystis* by qPCR. Similarly, increasing the number of qPCR replicates from three to nine led to 4-fold improvements in the probability of detecting DNA from known numbers of endospores that were spotted onto tape strips and extracted and amplified. Similar improvements in test sensitivity may be possible if new qPCR primers and probes that target multi-copy ribosomal genes rather than single copy nuclear genes like the cerato-platinin gene (Heller and Keith 2018) are developed. Estimates of detection limits for identifying *Ceratocystis* in air samples may be possible by combining individual probabilities for each step in the diagnostic process (Davis *et al.* 2018). For example, reduced test sensitivity due to a significant loss of DNA during the extraction may be corrected by increasing number of samples that are extracted and tested. Similarly, increasing the number of qPCR replicates may decrease stochastic probabilities of missing detections because

of low DNA template copy number. A more quantitative approach to analysis of environmental samples will help to reduce the probability of Type II errors related to false negative results.

While we demonstrated that airborne movement of potentially infective beetle frass can take place over relatively short distances, both viability of this frass and the likelihood that it can initiate new infections in healthy trees that have been recently wounded by human activity, feral animals, or high winds remains unknown. Additional work to determine viability of material collected on tape strips and distance that infectious frass and other particulates can move in the wind will be important for assessing airborne dispersal and transmission in this pathosystem.

ACKNOWLEDGEMENTS

We thank Don and Wendie Sherrad and Nick Manoukis and Lori Carvalho for generous access to field sites, Nicolette Thomas, Mali'o Kodis, and William Watcher-Weathermax for assistance in the lab, Lisa Keith and Wade Heller for arranging use of facilities at USDA-ARS-DKI-PBARC, Tom Harrington for use of Rotorod® Samplers and supplies, and Flint Hughes and Marc Hughes for helpful reviews of the manuscript. This work was funded in part by the U.S. Geological Survey Natural Resource Protection Program, the U.S. Department of Interior and U.S. Department of Agriculture joint Service First Program, and the U.S. Geological Survey Ecosystems Mission Area. Editorial assistance was provided by J. Rowe. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

LITERATURE CITED

Aizenberg, V., T. Reponen, S. Grinschpun, and K. Willeke. 2000. Performance of Air-O-Cell, Burkard, and Button Samplers for total enumeration of airborne spores. American Industrial Hygiene Association Journal 61: 855–864.

Banks, L. and F. Di Giovanni. 1994. A wind tunnel comparison of Rotorod® and Samplair pollen Samplers. Aerobiologia 10: 141–145.

Barnes, I., A. Fourie, M. J. Wingfield, T. C. Harrington, D. L. McNew, L. S. Sugiyama, B. C. Luiz, W. P. Heller, and L. M. Keith. 2018. New *Ceratocystis* species associated with rapid death of *Metrosideros polymorpha* in Hawai`i. Persoonia 40: 154–181.

Bittner, T. D., A. E. Hajek, A. M. Liebhold, and H. Thistle. 2017. Modification of a Pollen Trap Design to Capture Airborne Conidia of *Entomophaga maimaiga* and Detection of Conidia by Quantitative PCR. Applied and Environmental Microbiology 83(17): e00724-17.

Brown J. 1997. Airborne inoculum, pp. 207–218. *In* J. F. Brown and H. J. Ogle, (editors). Plant pathogens and diseases. Rockvale Publications, Armidale, NSW, Australia.

Brown, J. K. M. and M. S. Hovmøller. 2002. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. Science 297: 537–541.

Carvalho E., C. Sindt, A. Verdier, C. Galan, L. O'Donoghue, S. Parks, and M. Thibaudon. 2008. Performance of the Coriolis air sampler, a high-volume aerosol-collection system for quantification of airborne spores and pollen grains. Aerobiologia 24: 191–201.

Chen, W., S. Hambleton, K. A. Seifert, O. Carisse, M. S. Diarra, R. D. Peters, C. Lowe, J. T. Chapados, and C. A. Lévesquea. 2018. Assessing Performance of Spore Samplers in Monitoring Aeromycobiota and Fungal Plant Pathogen Diversity in Canada. Applied and Environmental Microbiology 84(9): e02601-17.

Crisp, H. C., R. A. Gomez, K. M. White, and J. M. Quinn. 2013. A side-by-side comparison of Rotorod® and Burkard pollen and spore collections. Annals of Allergy, Asthma and Immunology 111: 118–125.

Davis, A. J., K. E. Williams, N. P. Snow, K. M. Pepin, and A. J. Piaggio. 2018. Accounting for observations processes across multiple levels of uncertainty improves inference of species distributions and guides adaptive sampling of environmental DNA. Ecology and Evolution 8: 10879–10892.

Dedeurwaerder, G., M. Duvivier, S. M. Mvuyenkure, M. E. Renard, V. Van Hese, G. Marchal, J. M. Moreau, and A. Legrève. 2011. Spore traps network: a new tool for predicting epidemics of wheat yellow rust. Communications in Agricultural and Applied Biological Sciences 76: 667–670.

Di-Giovanni, F. 1998. A review of the sampling efficiency of rotating-arm impactors used in aerobiological studies, Grana 37: 164–171.

Eaton, S., C. Zúniga, J. Czyzewski, C. Ellis, D. R. Genney, D. Haydon, N. Mirzai, and R. Yahr. 2017. A method for the direct detection of airborne dispersal in lichens. Molecular Ecology Resources 18: 240–250.

Engelbrecht, C. J., T. C. Harrington, and A. Alfenas. 2007. *Ceratocystis* wilt of cacao—A disease of increasing importance. Phytopathology 97: 1648–1649.

Eversmeyer, M. G., C. L. Kramer, and T. I. Collins. 1975. Three suction-type spore samplers compared. Phytopathology 66: 62–64.

Keith, L. M., R. F. Hughes, L. S. Sugiyama, W. P. Heller, B. C. Bushe and J. B. Friday. 2015. First report of Ceratocystis Wilt on Ōhi`a (*Metrosideros polymorpha*). Plant Disease 99: 1276.

Frenz, D. A. and J. C. Elander. 1996. A calibration program for Rotorod® samplers. Annals of Allergy, Asthma & Immunology 76: 245–246.

Frenz, D. A., Scamehorn, R. T., Hokanson, J. M., L. W. Murray. 1996. A brief method for analyzing Rotorod® samples for pollen content. Aerobiologia 12: 51–54.

Grosclaude, C., R. Olivier, J. C. Pizzuto, and C. Romiti. 1991. Etude experimentale du transport de I'inoculum de *Ceratocystis fimbriata* f. *platani* par I'eau d'une riviere. European Journal of Forest Pathology 21: 168–171

Han, T., H. Zhen, D. E. Fennell, and G. Mainelis. 2015. Design and evaluation of the field-deployable electrostatic precipitator with superhydrophobic surface (FDEPSS) with high concentration rate. Aerosol and Air Quality Research 15: 2397–2408.

Harrington, T. C. 2013. *Ceratocystis* Diseases. Chapter 11, pp. 230–255 *in* P. Gonthier and G. Nicolotti (editors). Infectious Forest Diseases. CABI, Wallingford, UK.

Heffer, M. J., J. D. Ratz, J. D. Miller, and J. H. Day. 2005. Comparison of the Rotorod® to other air samplers for the determination of Ambrosia artemisiifolia pollen concentrations conducted in the Environmental Exposure Unit. Aerobiologia 21: 233–239.

Heller, W. P; Keith, L. M. 2018: Real-time PCR assays to detect and distinguish the Rapid `Ōhi`a Death pathogens *Ceratocystis lukuohia* and *Ceratocystis huliohia.* Phytopathology (in press).

Hirst, J. M. 1952. An automatic volumetric spore trap. Annals of Applied Biology 39: 257–265.

Iton, E. F. 1959. Studies on a wilt disease of cacao at River Estate. A report on cacao research, 1957–1958, Imperial College of Tropical Agriculture, Trinidad, pp. 55–64.

Iton, E. F. 1961. Studies on a wilt disease of Cacao at River Estate. II. Some aspects of wind transmission. pp. 47–58 *in* Report on Cacao Research, 1959–60. Imperial College of Tropical Agriculture. University of the West Indies, St. Augustine, Trinidad.

Kaye P.H., W. R. Stanley, E. Hirst, E. V. Foot, K. L. Baxter, and S. J. Barrington. 2005. Single particle multichannel bio-aerosol fluorescence sensor. Optics Express 13: 3583–3593.

Lei, Y., Z. Yao, and D. He. 2018. Automatic detection and counting of urediniospores of *Puccinia striiformis f. sp. tritici* using spore traps and image processing. Scientific Reports 8:13647.

Levetin, E., C. A. Rogers, and S. A. Hall. 2000. Comparison of pollen sampling with a Burkard spore trap and a Tauber trap in a warm temperate climate. Grana 39: 294–302.

Luchi, N. L. Ghelardini, L. Belbahri, M. Quartier, and A. Santini. 2013. Rapid detection of *Ceratocystis platani* inoculum by quantitative real-time PCR assay. Applied and Environmental Microbiology 79: 5394–5404.

Núñez, A., G. Amo de Paz, A. Rastrojo, A. M. García, A. Alcamí, A. Montserrat Gutiérrez-Bustillo, and D. A. Moreno. 2016. Monitoring airborne biological particles in outdoor atmosphere. Part 1: Importance, variability and ratios. International Microbiology 19: 1–13.

Mahaffee, W. F., and R. Stoll. 2016. The ebb and flow of airborne pathogens: monitoring and use in disease management decisions. Phytopathology Review 106: 420–431.

Moller, W.J.; DeVay, J.E., (1968). Carrot as a species-selective isolation medium for *Ceratocystis fimbriata*. Phytopathological Notes 58,123–126.

Mortenson, L. A., R. Flint Hughes, J. B. Friday, L. M. Keith, J. M. Barbosa, N. J. Friday, Z. Liu, and T. G. Sowards. 2016. Assessing spatial distribution, stand impacts and rate of *Ceratocystis fimbriata* `ōhi`a (*Metrosideros polymorpha*) mortality in a tropical wet forest, Hawai`i Island, USA. Forest Ecology and Management 377:83–92.

Ocasio-Morales, R. G., P. Tsopelas, and T. C. Harrington. 2007. Origin of *Ceratocystis plantani* on Native *Platanus orientalis* in Greece and its impacts on natural forests. Plant Disease 91: 901–904.

R Core Team. 2017 R: A Language and Environment for Statistical Computing. https://www.R-project.org/.

Rogers, S. L., S. D. Atkins and J. S. West. 2008. Detection and quantification of airborne inoculum of *Sclerotinia sclerotiorum* using quantitative PCR. Plant Pathology 58: 324–331.

Roy, K., C. P. Ewing, M. A. Hughes, L. Keith, and G. M. Bennett. 2018. Presence and viability of *Ceratocystis lukuohia* in ambrosia beetle frass from Rapid ʻŌhiʻa Death-affected *Metrosideros polymorpha* trees on Hawai'i Island. Forest Pathology (In Press).

Schneider R.W., E. Durr E., and C. G. Giles. 2007. A new spore trap that utilizes electrostatic deposition and scanning electron microscopy. Phytopathology 97: S105.

Schweigkofler, W., K. O'Donnell, and M. Garbelotto. 2004. Detection and Quantification of Airborne Conidia of *Fusarium circinatum*, the Causal Agent of Pine Pitch Canker, from Two California Sites by Using a Real-Time PCR Approach Combined with a Simple Spore Trapping Method. Applied and Environmental Microbiology 70: 3512–3520.

Souza, A. G. C., Maffia, L. A., Murta, H. M., Alves, Y. H., Pereira, R. M., & Picanço, M. C. (2013). First report on the association between *Ceratocystis fimbriata*, an agent of Mango Wilt, *Xyleborus affinis*, and the sawdust produced during beetle colonization in Brazil. Plant Disease 97: 1116–1116.

Systat. 2011. Version 11.00.01. Systat Software, San Jose, CA.

von Qualen, R. and X. B. Yang. 2006. Spore traps help researchers watch for soybean rust. Integrated Crop Management News 1308. http://lib.dr.iastate.edu/cropnews/1308.

West, J. S. and R. B. E. Kimber. 2015. Innovations in air sampling to detect plant pathogens. Annals of Applied Biology 166: 4–17.

Wingfield, M. J., I. Barnes, Z. Wilhelm de Beer, J. Roux, B. D. Wingfield, and S. J. Taerum. 2017. Novel associations between opiostomatoid fungi, insects and tree hosts: current status—future prospects. Biological Invasions 19: 3215–3228.