COLLECTING SAMPLES FROM OHIA TREES SUSPECTED OF BEING INFECTED WITH *CERATOCYSTIS* (RAPID OHIA DEATH)

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Background: Two newly introduced species of the plant pathogen, *Ceratocystis,* referred to as species A and species B, are known to cause the disease known as Rapid Ohia Death in ohia trees on Hawaii Island. For both species, the fungus is known to grow within the sapwood, but not on the surface of the trees (i.e. bark surface).

Sanitation: Precautionary measures should be taken to prevent the transmission of the fungus to new hosts via contaminated cutting implements, gloves, hands, etc., by spraying with 70% isopropanol (rubbing alcohol) or 70% ethanol or 10% bleach (freshly mixed solution). Sawdust created when cutting into diseased trees contains a high number of viable spores; efforts should be made to contain and/or decontaminate any sawdust. Sawdust can be caught on tarps spread on the ground around the tree and collected for disposal; wetting the tarp slightly helps to catch sawdust. Felling trees on no wind/slightly wet days will help minimize airborne movement of infectious sawdust. Chainsaws must be thoroughly cleaned, including removing the bar and chain, to remove residual sawdust and disinfect surfaces between samples. It is important to prevent cross-contamination of samples by disinfecting tools, gloves, etc. before starting and after finishing collection of each sample. Testing methods include both culture-based techniques and molecular methods (DNA testing). Only culture-based techniques will determine viability of the fungus; DNA tests might additionally reveal the presence of fungus that is no longer alive due to heating, other treatments, or the length of time a tree has been dead.

Range of 'typical' staining/discoloration observed in ohia infected with either *Ceratocystis* species:

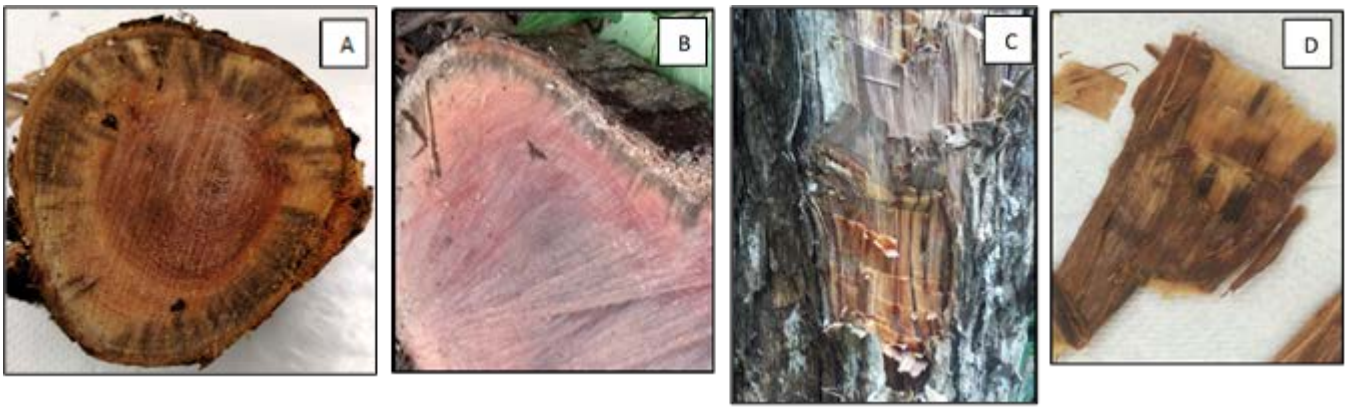

Figure 1. Typcial symptoms of ohia wood associated with *Ceratocystis* species A: an outward radiating pattern (starburst) of discoloration from stem cross-sections ("cookies") (A,B) and vertical streaks under the bark (C,D). Streaks are usually brown to black in color.

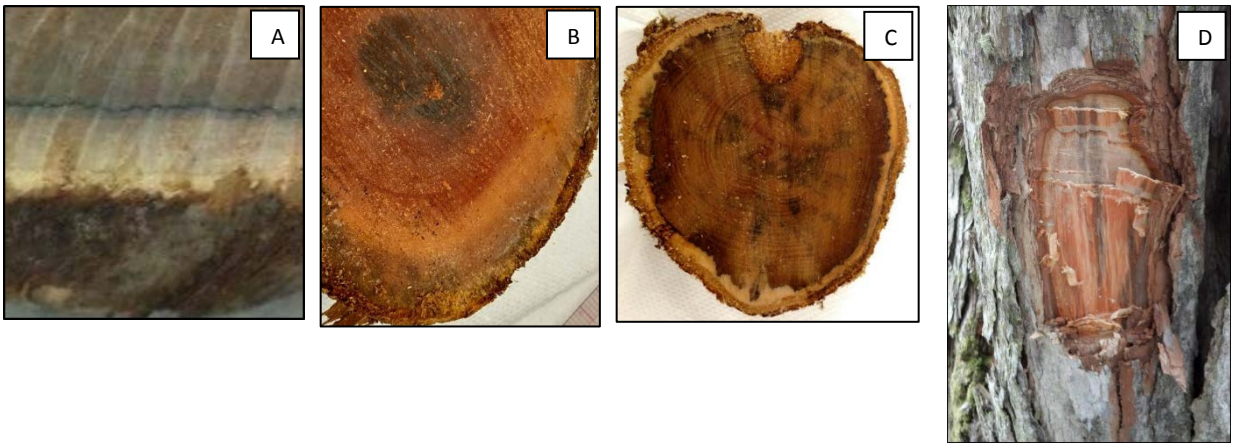

Figure 2. Typical symptoms of ohia wood associated with *Ceratocystis* species B: thin black lines of discoloration along the outer edge of cross-sections ("cookies") underneath the bark layer (A,B,C) and vertical streaks under the bark (D). Streaks are usually brown to grey-black in color.

Possible collection methods:

FOR SUSPECT TREES IN NEW LOCATIONS, ESPECIALLY HEALTHY FORESTS, WE RECOMMEND THAT INITIAL SAMPLES ARE COLLECTED BY THE DRILLING AND/OR SLASHING WITH A HATCHET OR AXE TECHNIQUES. THE USE OF A CHAINSAW CAN DISPERSE LARGE AMOUNTS OF INFECTIOUS SAWDUST. CONTAINMENT AND DECONTAMINATION ARE EXTREMELY DIFFICULT!

1. Drilling. *Pros*: Drilling is quick to perform and easy to make composite samples from many points on the same tree. Drilling makes a good first test. *Cons*: The sampling is blind. It is very unlikely that discoloration will be detected in shavings. Sampling is limited to places the collector can reach.
    a. Method notes:
        i. Use a 5/16" drill bit in a cordless drill. Use a portable propane torch (e.g. Bernzomatic) to flame sterilize drill bit between trees. Sterilizing bits with heat rather than alcohol destroys the DNA of the fungus and prevents cross-contamination, whereas alcohol will kill the fungus but may not destroy the DNA.
        ii. Drill at least 2" deep into at least 4 places (N-E-S-W) around breast height from a single tree, or from a suspect branch. Collect shavings into a plastic bag for testing.
        iii. Brush any residual wood shavings from the drill bit before sterilizing by flame or bleach/alcohol method. If working in an area where fire risk precludes use of the torch, keep a stock of clean, wrapped drill bits (to prevent cross contamination) on hand and change between samples. Clean hands and/or change gloves between samples.

2. Slashing by hatchet or axe. *Pros*: Slashing offers some ability to look for staining or discoloration, which is usually apparent as "streaking" (Figures 1c & 2d). Slashing disperses far less inoculum than felling the tree and cutting sections with a chainsaw would. *Cons*: As with drilling, a limited amount of the tree can be accessed by this method. Slashing leaves open wounds that could attract wood-boring insects. If an infection started in the crown of the tree, it might be missed by drilling or slashing at breast height.
   See YouTube video for demonstration: https://www.youtube.com/watch?v=DKlRoisstD0
   a. Method notes:
      i. Slash multiple places on the trunk to look for streaking underneath the bark. It may be necessary to cut a deep wound at least half an inch to detect the streaking. Collect wood pieces (not bark) and place in a plastic bag.
      ii. Disinfect hatchet or axe with 70% alcohol or a freshly-mixed 10% bleach solution between samples. Clean hands and/or change gloves between samples.

3. Beetle boring dust (frass). *Pros*: Collecting beetle boring dust or frass is the least invasive method and is quick to perform and easy to make composite samples from many points on the same tree. Like drilling, collecting beetle boring dust or frass is a good first test. *Cons*: The sampling is blind. It is very unlikely that discoloration will be detected in beetle boring dust. Boring dust and frass is not found on all trees nor at all stages of infection.
   a. Method notes:
      i. Collect insect boring dust or frass (Figure 3) in a plastic bag or a clean capped vial or conical tube for testing.
      ii. If insect boring dust/frass is held up in the bark, peel back or loosen bark and frass should pour out.
      iii. Clean hands and/or change gloves between samples.

Figure 3. Bark and ambrosia beetle boring dust (frass) (within red circles) that collects in trunk bark cracks and branch crotches.

4. Felling the tree and taking cross sectional samples ("cookies"). *Pros*: Felling the tree offers the greatest ability to assess staining or discoloration associated with either species of *Ceratocystis*

(Figures 1 & 2). Many cookies can be cut along the length of the tree to detect localized infections. *Cons*: Felling trees is dangerous and should only be attempted by trained and experienced personnel. Felling is very labor intensive. Sawdust from chain-sawing infected trees has been shown to contain live *Ceratocystis* spores and can become broadly dispersed in the felling location. Felling trees in a dense stand can injure neighboring, healthy trees and create the possibility of more infections.

a. Method notes:
    i. Fallers should wear safety equipment, including chainsaw chaps, boots, and eye and ear protection. Take all the usual precautions in felling trees: make sure the area is clear and all other personnel are out of the range of the falling tree. Make sure to have two escape routes to leave as the tree comes down. Do not fell trees with large dead branches in the crown ("widowmakers").
    ii. Make attempts to contain and/or decontaminate sawdust by sampling on calm, wet days and collecting and removing sawdust with tarps.
    iii. Once the tree is on the ground, cut thin cookies, under 1" thick, and place in plastic bags. Look for signs of staining in the sapwood (xylem) (Figures 1 & 2). Take one sample every 4 to 6 feet along the stem. If multiple cookies from the same tree are simultaneously submitted, mark the samples to indicate their relationship to each other "Tree 1, cookie A", "Tree 1, cookie B." If possible, provide a map of cookie location on the tree.
    iv. Sawdust can also be submitted as a sample. Do not mix the sawdust with dirt or other non-ohia particles.
    v. Thoroughly clean chain saw by taking off the bar and chain, removing all loose sawdust, and disinfecting with 70% alcohol or 10% bleach between trees. Clean hands and/or change gloves between samples.

Disinfecting Tools: Brush or wipe tools free of wood particles. Spray cleaned tool surface with 70% alcohol (isopropanol or ethanol) or a freshly made 10% bleach solution and allow to soak at least 5 minutes; rinse with clean water or allow to air-dry before use. A portable propane torch can also be used to heat particle-free, metallic surfaces (hatchet blades and drill bits) to kill infectious propagules and allow to air dry until cool to touch. Always disinfect tools between the sampling of multiple trees.

Photo Record: Take pictures of the individual tree prior to sampling and pictures of the tree slash sites and/or cookies to record any internal discoloration. Discoloration may darken once exposed to air and discoloration often becomes less obvious upon storage. Photographs of both the entire tree and affected leaves and branches are useful.

Labeling: Label any bags with a specific name for the site. GPS points are ideal if available, otherwise street addresses help. Include the collector's name, the date of collection and the location. Complete the sample submission form.

Delivery: Drop off samples to your local Hawaii Department of Agriculture Plant Quarantine Branch office or, if in Hilo, the USDA ARS office located at 64 Nowelo St. It is important that samples arrive in

the lab as quickly as possible after collection, ideally within 48 hours, although slightly longer storage periods are okay if refrigerated.

Hawai‘i Department of Agriculture Plant Quarantine Branch Locations

Oahu Plant Quarantine Branch 1849 Auiki Street Honolulu, HI 96818 (808) 832-0566	**Maui or Molokai** Plant Quarantine Branch 635 Mua Street Kahului, HI 96732 (808) 873-3962
Kauai Plant Quarantine Branch 4398A Pua Loke Street Lihue, HI 96766 (808) 241-7135	**Hawai‘i Island** Plant Quarantine Branch 16-E Lanikaula Street Hilo, HI 96720 (808) 974-4141

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