

Introduction

Rapid 'Ōhi'a Death (ROD) caused by a new pathogen called *Ceratocystis* is killing thousands of 'ōhi'a (*Metrosideros*) trees (<u>rapidohiadeath.org</u>). It poses a serious risk to the health and future of native forests statewide. Research, new quarantine rules, and increased sanitation measures are underway to slow the spread of the disease on Hawai'i Island and reduce the risk of spread to other islands. As part of the <u>2017-2019 ROD Strategic Response Plan</u>, 'ōhi'a seed collections are urgently needed statewide. Seeds of 'ōhi'a can be secured in long-term germplasm storage to mitigate the potential loss of genetic diversity and provide appropriate plant material for restoration, watershed rehabilitation, and saplings for research and testing for resistance or tolerance to the fungal disease.

PLEASE CONTACT ONE OF THE FOLLOWING HSBP SEED BANK FACILITIES BEFORE MAKING OR SENDING COLLECTIONS

Location	Seed Bank Facility	Contact	Email	Phone
Oʻahu	u Lyon Arboretum Seed Conservation Laboratory (LASCL) Anna Sugiyama <u>asugi@hawaii.edu</u>		8089880469	
Hawai'i	Hawaii Island Seed Bank (HISB)	Jill Wagner	jillwagner3@icloud.com	8083252377
Hawai'i	Ulu Lehulehu Seed Bank (U.S. Forest Service, Hilo)	Ulu Lehulehu Team Kainana Francisco	ululehulehu@gmail.com 8088522652	
Kaua'i	National Tropical Botanical Garden (NTBG) Seed Bank & Lab	Dustin Wolkis	dwolkis@ntbg.org 8088002704	
Kaua'i	Division of Forestry and Wildlife (DOFAW) Kaua'i Seed Bank	Adam Williams	adam.m.williams@hawaii.gov	
Maui	Maui Nui Botanical Gardens	Tamara Sherril	info@mnbg.org	8082492798
All	Hawai'i Seed Bank Partnership (HSBP)	Marian Chau	mmchau@hawaii.edu	8083480823

Collaboration

A partnership is needed to make collections, store the seeds, organize the records, and communicate updates. Public agencies, NGOs, and private landowners are being asked to contribute resources to make the collections. The Hawai'i Seed Bank Partnership is seeking support for transporting, cleaning, and storing the collections, and will provide record keeping and communications on project updates. The following tools are available to standardize best practices statewide:

- 'Öhi'a Seed Collection Partnership of agencies, landowners, and other partners engaged in this effort
- 'Ohi'a Collection & Transport Protocols for making and transporting collections
- 'Ōhi'a Collection Form for recording collection information
- 'Ohi'a Collection Map indicating where collections have been made and for targeting future collections
- 'Ōhi'a Collection Needs to communicate updates

Ownership of Collections

The ownership and conditions for releasing any collections of 'ōhi'a seeds submitted to HSBP facilities must be specified for each collection. The <u>collectors</u> are responsible for permits, permissions, and any other approval needed to make, transport, and submit seed collections to HSBP facilities. Please be prepared to select from the following options in the 'Permission to Release Collection' section of the <u>'Ōhi'a Collection Form</u> to guide future use of the collection:

- o Permission REQUIRED from Landowner/Manager- Owner/manager will approve all requests for seeds (Default)
- o Permission REQUIRED from Collector Agency- The collector agency will approve all requests for seeds
- Permission PRE-APPROVED for Certain Groups- List
- o NO Permission Required- The seeds may be released for any non-commercial conservation or educational use

Sanitation

The fungus that causes ROD has both sticky spores that stick to debris from wood-boring insects and hard coated spores that survive in soil, on live trees, and in harvested 'ōhi'a wood. The spores may lay dormant for months or even years. It has been detected outside of Hawai'i Island now, so following sanitation protocols – even in areas where it is not known – is critical to prevent spread. Be sure to thoroughly clean your tools and shoes using at least 70% alcohol, and wash your clothes and all other gear in hot water and detergent before hiking in a new location. Consider designating separate field gear for work in each island or mountain range to minimize risk of spreading spores. If you drive into 'ōhi'a forest, pressure-



































'Ōhi'a Collection and Transport Protocols

Sampling Strategy

# 'Ōhi'a TAXA	# TREES	# CAPSULES
14	Up to 10,000 trees from each taxon	Minimum: 25 (5,000 seeds)
Including varieties	Representing all populations statewide	Maximum: 75 (15,000 seeds)
TOTAL	140,000 trees	700 Million – 2.1 Billion Seeds!

- 1) **Only collect from wild populations**. In order to achieve collections that are genetically diverse and representative of each taxon, 10,000 individual trees will be sampled from across its range. Outlier trees growing in unique conditions should be sampled. To provide seeds that can be used to detect resistance of certain individuals to ROD, collections from *individual trees must be kept separately*. Combining collections into a bulk sample should be avoided whenever possible to preserve the highest conservation value, but can be included if collectors are limited by time or supplies.
- 2) **#Subpopulations (seed zones)**: The range of each taxon has been divided into 'subpopulations' or 'seed zones' to delineate the areas in which seeds should be used for restoration projects. These zones define an area where plant materials can be transferred with little risk of being poorly adapted to their new location (<u>BLM SOS</u>). A map of seed zones for each taxon can be found here: (<u>'Ōhi'a Collection Map</u>). Some taxa occur on a single mountain range while others are found on multiple islands. In order to capture and preserve this variation, collections are needed from each seed zone.
- 3) #Individual Trees: The target number of individuals to be sampled from each seed zone ranges from 280 for a taxon with a population in many zones, to 2,500 trees for one with a narrow range. The current needs for collections in each zone are tracked here: 'Ohi'a Collection Needs. The goals will be adjusted as surveys on the ground help to define the seed zones.
- 4) **#Seeds**: *Collect seeds from 25-75* <u>ripe individual capsules</u> from each tree. Estimates from previous collections suggest that 25 ripe full capsules will have at least 5,000 seeds. If the capsules have already started to open, more need to be collected to meet our minimum goal of 5,000 seeds per tree. With a collection of 50 capsules (10,000 seeds), the collection will be split into two accessions in two HSBP facilities. With a collection of 75 capsules (15,000 seeds), the collection will be split at three facilities. Whenever possible, larger collections of 75 capsules from each tree are encouraged to provide adequate plant material for future research. If you remove seeds in the field, each 5000 seeds are about the volume of a quarter (coin, see photo below).

Making and Cleaning Collections

When collecting in an area where ROD may be present, the seeds <u>must</u> be removed from the capsules and the discarded material left on site to reduce the chance of transporting fungal spores on or in the collection material.

Carefully remove ripe capsules from the tree and transfer to a dry paper envelope (but check first to ensure that envelopes do not have small openings in the corners that let tiny 'ōhi'a seeds escape). A plastic bag could be used for the initial collection (Video Example), but the seeds must be transferred to paper at the end of the day or they will begin to mold and suffocate. When collecting capsules in areas where ROD is known or suspected, the seeds should be removed from the capsules in the field and all other debris discarded on site. If capsules are collected and brought in for processing, seeds can be removed by placing the cluster of capsules face down on a clean tray in a dry area, away from sun and wind, for about a week. Within this time, most of the capsules will open completely and release seeds. A toothpick or needle can be used to gently remove any seeds that are still stuck inside the capsules. Remove any large pieces of capsules/stems by hand. Use a sieve or kitchen strainer to separate seeds from smaller debris before submitting to a HSBP facility (standard mesh sizes #18 or #20 work best to allow all seeds through and capture most debris). A small amount of debris about the same size as seeds is acceptable for seed bank submission. These five pieces of information are required to physically accompany each collection to the seed bank: Collector Name, Collection Date, Type of 'Ōhi'a, Plant Number, Location. Please write this basic info on collection bags and paper packets/envelopes submitted to the seed bank.































'Ōhi'a Collection Pictures

(Photos by Forest and Kim Starr (starrenvironmental.com) & Lyon Arboretum)







Mature seed capsules (ready)



Empty seed capsules (too late)









Three examples of mature 'ōhi'a capsules full of seeds ready to harvest

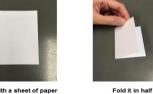
5,000 mature 'ōhi'a seeds

Transport and Shipping

To prepare seeds for transport, dry capsules in a cool dry area and transfer dry seeds to a well-sealed paper or glassine envelopes, or packets that can be made from paper (example on right). Label and seal each packet and pack into a padded shipping envelope or box. In order to successfully conserve the seeds, it is critical that collections be sent or delivered to a HSBP facility within a few days of collection for processing. The 'Ohi'a Collection Form must accompany each collection. Until more funding can be secured, collectors are responsible for packing and transport costs to HSBP facilities. Before making the collections, please contact the HSBP facility by email or phone (above) to coordinate delivery to ensure it will be quickly processed.



Start with a sheet of paper or ½, ¼, ⅓, sheet)





Fold in the two parallel open







Seed Bank	Accepting Collections from:	Address
Lyon Arboretum	Oʻahu, Maui, Molokaʻi, Lanai	3860 Manoa Rd. Honolulu, HI 96822 Attn: Marian Chau
Hawai'i Island Seed Bank	Hawai'i Island	P.O. Box 847, Kailua Kona, HI 96745 Attn: Jill Wagner
Ulu Lehulehu Seed Bank	Hawai'i	60 Nowelo St., Hilo, HI 96720 Attn: Ulu Lehulehu Seed Bank
National Tropical Botanical Garden	Kaua'i	3530 Papalina Rd. Kalaheo, HI 96741 Attn: Dustin Wolkis
Kauai Div. of Forestry and Wildlife	Kaua'i	4398-D Pua Loke St. Lihue, HI 96766 Attn: Denise Duenas
Maui Nui Botanical Garden	Maui, Molokaʻi, Lanaʻi	150 Kanaloa Ave. Kahului, HI 96732 Attn: Tamara Sherrill



'Ōhi'a Seed Collection Data Collection Instructions

This Mobile Webform is Available here: https://ee.kobotoolbox.org/x/#Yi0V.

	*Collection Date	
1	yyyy-mm-dd hh:mm	Enter the date and time the collection was made
2	*Collecting Agency DOFAW PEPP OANRP NPS TNC	Select from the list of agencies, or use 'Other' and enter the name of the collector on the next page
3	*Collector Name	Enter the full name of the collector
4	*Taxon Name UNKNOWN (MUST submit photo) Hybrid (MUST describe in Notes) macropus polymorpha var dieteri polymorpha var. glaberrima	Select the type of 'ōhi'a from this list. If unsure, select 'UNKOWN' and make sure to provide a picture of the underside of the leaf. If you suspect it is a hybrid, select 'Hybrid' and enter notes on the next page.
5	Taxon Notes/Description	Enter any notes on the type of 'ōhi'a or origin of the hybrid
6	*Which Hawaii Seed Bank Partnership facility will receive the seeds? Please make sure the Seed Bank knows you will be submitting seeds Lyon Arboretum National Tropical Botanical Garden Hawaii Island Seed Bank Oahu Army Natural Resources Program US Forest Service - Hilo	Select the Seed Bank which will be receiving the seed collection. If it is not on the list, use 'Other'
7	Island What Island are you on? Kauai Oahu Molokai Maui Lanai Hawaii	Enter the island where the collection was made. If not on the list, you are not in Hawai'i.
8	*Population Reference Code / Plant Number Enter a plant number (start with 1)	Enter the individual plant number for this collection. Start at 1, or enter the full Population Reference Code



9	*GPS Location GPS coordinates can only be collected when outside. latitude (x.y°) longitude (x.y°) altitude (m) accuracy (m) search for place or address Q	Use the button on the top left corner of the page to take a GPS point. Or, enter the latitude, longitude, and elevation in meters where the collection was made
10	Site Name / Directions	Provide a name of the area and/or directions to the collection location
11	*Number of Capsules Collected	Count or estimate the number of capsules collected
12	*Collections kept separate (Individual trees) or combined (Bulk) Separate Individual collections enable use for research and restoration Individual (recommended to ensure a high conservation value for future use) Bulk (not recommended for resistance testing or when taxon is unknown)	Indicate whether the collections from individual trees were kept separate, or if they were combined into a bulk collection
13	Fruit Maturity 75-100% Capsules Open 50-75% Capsules Open 25-50% Capsules Open	What percentage of the capsules on the tree you collected from were mature and open?
14	*Post-Harvest Ripening Seeds Removed from Capsules in the Field Post-Harvest Protocol Followed Seeds still in capsules (not recommended!)	Were the seeds removed from the capsules in the field, or after the collection was made following the post-harvest protocol?
15	*Is this collection authorized by the landowner? O YES O NO	Indicate whether the collection was authorized by the landowner























Landowner













16	Private (List landowner below) State of Hawaii (DOFAW, DHHL, etc) U.S.A. (Alational Park, PMS Parkers, DOD)	If 'Other', please explain on the next page.
	U.S.A. (National Park, FWS Refuge, DOD)	
17	*Permission to Release Collection NO Permission Required: Seed Bank can release for any conservation use PERMISSION REQUIRED from the Landowner/Manager PERMISSION REQUIRED from the Collection Agency Permission PRE-APPROVED for the groups listed below	Based on the stipulations in the collection permit, or discussions with the landowner, what permission does the seed bank need to release seeds? If unknown, use the second option.
18	List the Landowner/Manager or Collection Agency Authorized for Release & other Notes	If permission is 'PRE-APPROVED', indicate which groups have access
19	Permit #	If the collection is covered by a government permit, enter the permit number here
20	Enter Other Collector Names or Collector Number	Enter any other names or collector numbers associated with the collection
21	Photo Voucher REQUIRED when species or variety is unknown. Please take a picture of the underside of the leaf.	Take or attach a picture of the underside of the leaf
22	Photo Taken? YES NO (Photo is required if the species or variety is unknown)	Indicate whether a photo was taken or attached
23	*Is ROD known from this area? In the area you covered today, are there trees that have died because of ROD? YES NO	Is ROD known from the area where you collected?
24	*% of Trees with symptoms of ROD What % of trees in the area have died because of ROD?	If you entered 'YES' for #23, what percentage of the trees in the collection area have died?



25

































ROD	Notes/Desci	riptio

on

If you entered 'YES' for #23, provide any other notes on ROD

26	* Do you want to enter Habitat Characteristics? This information will help us guide the future use of your collections YES NO (will provide later)	Would you like to enter other information about where the collection was made to help guide its use?
27	Phenology Select any of the options below that describe the tree you collected from Vegetative (no flowers or fruit) Bud Flower	Describe the phenology of the tree collected from
28	Canopy Height How tall is the tree you collected from? <5 m 5-10 m	How tall is the tree you collected from?
29	Light Level How much light is there on the tree you collected? Full Sun 95% Partial Sun 50-95% Partial Shade 5-50%	How much light is there on the tree you collected?
30	Condition How healthy is the tree you collected from? Healthy Moderate Poor Dead	How healthy is the tree you collected from?
31	* Moisture Class What is the annual rainfall in this area? Dry <25" Dry-Mesic 25-50" Mesic 50-75"	What is the annual rainfall in the collection area?
32	*Aspect What direction is the slope facing in this area? North South East	Which direction is the slope facing in this area?



		a Changing World
	*Slope	
	How steep is the slope in this area?	
33	Flat 0-10	How steep is the slope in this area?
	Moderate 11-45	
	Steep 45-70	
	*Substrate	
	What is the substrate like in this area?	
34	Soil	What is the substrate like in this area?
	Rock	
	aʻa	

PDF Collection Form Instructions

Email coordinator@laukahi.org for the PDF form

A COLLECTION FORM IS REQUIRED FOR EACH COLLECTION. PLEASE USE THE WEBFORM ABOVE, OR SEND A HARDCOPY OR DIGITAL VERSION OF THE PDF FORM TO THE HSBP FACILITY WHERE THE SEEDS ARE SUBMITTED.

HSBP Seed Bank Use Only (Only on PDF form, not webform?)

- 1.1. Receiving Seed Bank- Facility that received the collection
- 1.2. HSBP Accession Code- Unique tracking number assigned by HSBP facility
- 1.3. Received From- Person who delivered the collection
- 1.4. Date Received- Date received by the Seed Bank facility

OWNERSHIP AND PERMISSIONS

- 2.1. Collector Agency- Organization/Group/Agency making collection
- 2.2. Primary Collector Name- Full name of the lead collector
- 2.3. Other Collector Names- Full name of other collectors
- 2.4. Collector Number- If applicable, enter the number assigned by the collector
- 2.5. Collection Date- Date the collection was made, if made over multiple days, enter last date
- 2.6. Private or Public Land- Select from: Private, State of Hawaii, or U.S. owned land
- 2.7. Landowner Authorized Collection- Check box to indicate the landowner authorized the collection
- 2.8. Permit#- Enter the DOFAW or other agency permit number for this collection
- 2.9. Permission to Release Collection- Set material transfer agreement by selecting one of the following:
- 2.9.1. NO Permission Required: HSBP can release the collection for any conservation use
- 2.9.2. Permission REQUIRED from the Landowner/Manager: Permission from the Landowner or Manager listed in the box below is required before releasing
- 2.9.3. Permission REQUIRED from the Collector Agency: Permission from the Agency listed below is required for release
- 2.9.4. Permission PRE-APPROVED for certain groups: HSBP can release to any groups listed in the box below. Permission required from the Landowner/Manager for any other groups
- 2.10. Ownership Notes: Any other direction or stipulations for withdrawal and release of the collection
- 2.11. List Landowner/Manager/Collector authorized for release- List persons or groups for the selection made above

TAXON INFORMATION

- 3.1. Taxon Name- Select the Metrosideros taxon from the list
- 3.2. Taxon Notes- Include any notes on hybridization, unique morphological traits, etc.

LOCATION INFORMATION) (Any questions on how to assign a location, contact Matthew Keir at laukahi808@gmail.com)

- 4.1. Location Name- Place name where collection was made (valley, stream, pu'u, district, mauna, etc.)
- 4.2. Island Code- Select the island where the collection was made from the list































- 4.3. PopulationAreaCode- Select the HRPRG Population Reference Area where the collection was made. Use this map (PopRef Map) and list (PopRefList) to determine the correct code
- 4.4. PopulationSiteCode- Indicate or assign a Population Site Code where the collection was made (A-Z)
- 4.5. Seed Zone- Use this map (SeedZoneMap) to indicate the seed zone where the collection was made

PHOTO VOUCHER INFORMATION

Photo vouchers should include at least: 1) tree including bark, 2) detail of underside of leaf blade, 3) flowers/capsules

- 5.1. Photo Taken- Indicate whether photos of the trees were taken
- 5.2. File Name- Assign a file name so the collections can be linked with the photos

RAPID 'ŌHI'A DEATH

- 6.1. CHECK BOX- If the collections were made in an area where trees are suspected to be dying from ROD
- 6.2. % Trees Infected- Choose from the list to indicate the level of infestation at the collection site
- 6.3. Notes- Include notes to caution or guide future use of the collection for resistance testing or reforestation

HABITAT INFORMATION

- 7.1. Phenology (Vegetative, Bud, Flower, Immature Fruit, Mature Fruit)- Estimate % of trees in each category by decimal (50% = 0.5, 100% = 1), the total can exceed 100% when fruiting and flowering at the same time
- 7.2. Condition (Healthy, Moderate, Poor)- Estimate the % in each category by decimal (50% = 0.5, 100% = 1)
- 7.3. Light level (Full Sun, Partial Sun, Partial Shade, Deep Shade)- Indicate the light level in the immediate environment of the plant. Full sun, >95% of the day in direct sunlight, partial sun 50-95% of the day in direct sun, partial shade 5-50% of the day in direct sun, deep shade 0-5% of the day in direct sun and enter the decimal (50% = 0.5, 100% = 1)
- 7.4. Moisture- Select the moisture class where the collection was made from the list
- 7.5. Maximum Canopy- Select the maximum canopy height where the collection was made from the list
- 7.6. Aspect- Select the direction of the slope at the location (Flat, N, NW, NNW, etc.)
- 7.7. Slope- Select the slope of the ground where the collection was made
- 7.8. Substrate- Select the substrates present where the collection was made

SEED COLLECTION INFORMATION

- 8.1. Fruit Maturity- What % of the capsules collected are open
- 8.2. Post-Harvest Processing- Indicate if seeds were removed from the capsules in the field or post-harvest

INDIVIDUAL PLANT INFORMATION (For a collection of a single or several individual trees)

- 9.1. Plant #- Individual plant number
- 9.2. Plant Tagged- Indicate whether the plant was tagged (yes/no)
- 9.3. Height (m)- Estimate the height of each tree in meters
- 9.4. Latitude (Deg/Min/Sec)- Enter the Latitude of each tree in Degrees, Minutes, Seconds
- 9.5. Longitude (Deg/Min/Sec)- Enter the Longitude of each tree in Degrees, Minutes, Seconds
- 9.6. Enter the Universal Transverse Mercator (UTM) Zone- 05 for Hawaii Island, 04 for other main islands
- 9.7. UTM (north)- Enter the UTM northing coordinate
- 9.8. UTM (east)- Enter the UTM easting coordinate
- 9.9. Elevation- Enter the elevation in meters
- 9.10. Number of Ripe Capsules- Enter the number of capsules collected from each tree
- 9.11. Notes- Enter notes for each plant

BULK COLLECTION INFORMATION (Please be certain that collections are only combined from trees of the same taxon)

- 10.1. Latitude- Enter the Degrees, Minutes, Seconds
- 10.2. Longitude- Enter the Degrees, Minutes, Seconds
- 10.3. UTM- Enter the UTM Zone and coordinates















- 10.4. Location Notes- How do coordinates represent where the collection was made (i.e. middle of population, north end)
- 10.5. # Trees Observed- Estimate the number of trees in the collection area
- 10.6. # Trees Collected- Indicate how many trees the seeds were collected from
- 10.7. Area Covered (sq.km.)- Estimate the area in square kilometers where the collections were made
- 10.8. Photo Voucher Name- Assign a voucher code or file name so the collections can be linked with the photos if needed