



**Tropical Hardwood Tree Improvement  
& Regeneration Center**

# 2025 ANNUAL REPORT

**A Collaborative Research, Development, Education, and Extension Center for  
Tropical Hardwood Stewardship**

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## MISSION

The Tropical HTIRC mission is to advance the science and application of tree improvement, management, and protection to improve tropical hardwood forests, with emphasis on the Hawaiian Islands.





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## Director's Letter



## Aloha Kākou!

We are pleased to share our 2025 Tropical HTIRC Annual Report, the eighth annual report produced by our center to help promote communications and highlight each year's progress and developments.

During 2025, we continued to implement our 2022-2026 Tropical HTIRC strategic plan, which outlines our strategic objectives in research / development, education, extension, and capacity building. Our program focuses on reforestation and restoration of native Hawai'i trees to promote their commercial, cultural, and ecological

values. Tropical HTIRC began by working with koa, which remains an important foundation of Hawai'i forest research and management. Our projects have since diversified to include other species that help meet evolving needs in Hawai'i. There is new commercial and restoration interest in 'iliahi, the native Hawaiian sandalwood, which was exploited centuries ago. 'Ōhi'a is the most common native tree in the Hawaiian Islands, which is now threatened by Rapid 'Ōhi'a Death.



Field tours at Terraformation's 'Ōhi'a Lani at Waikoekoe restoration site near Waimea (top) and Kaiāulu Pu'uwa'awa'a, a community-based subsistence forest area at the Pu'uwa'awa'a Forest Reserve (opposite) during our Tropical HTIRC annual advisory committee meeting in February 2025.

Educating and training students is a cornerstone of our program. In 2025, we continued to work with an outstanding group of graduate students (see *bios below*). Highlights of the year included two successful Tropical HTIRC PhD student defenses at the University of Hawai'i at Mānoa: Pandu Wirabuana's dissertation, "*Mixed stand management between 'iliahi and host plants in Hawaiian-restored tropical dry forests*" and Solomon Champion's dissertation, "*Population genetic forestry and phylogenomics of Hawaiian sandalwoods*". Many of our students wish to continue with career trajectories dedicated to the conservation and management of Hawai'i forests. We are excited to welcome three new Tropical HTIRC graduate students in fall of 2026, two of which were born and raised on O'ahu. We also continued the Papa Noi'i

Lā'au Hawai'i (Hawaiian Plant Research Class) project, which engages local high school students in forest restoration research in Hawai'i.

Another highlight of 2025 was welcoming Jennipher Himmelmann to Tropical HTIRC (see *bio below*). Jennipher is an Urban and Community Forestry Resource Assistant, who is based at the USDA Forest Service Institute of Pacific Island Forestry in Hilo. She will be helping with administrative logistics of our project management and monitoring, as well as communications to help reach a wider audience. We are grateful to Miranda Hutten and Christian Giardina (USDA Forest Service) for facilitating this opportunity. Welcome Jennipher!

Tropical HTIRC works collaboratively with partners to develop projects that address the challenges in managing and restoring



**Pandu Wirabuana, with advisor Travis Idol and committee member JB Friday, after successful defense of his dissertation at the University of Hawai'i at Mānoa.**

Hawai'i forests. Our Tropical HTIRC graduate students have ongoing projects with many of our partners including Department of Hawaiian Home Lands, State of Hawai'i Division of Forestry and Wildlife (DOFAW), Hāloa 'Āina, Kealakekua Mountain Reserve, and Parker Ranch. We also deeply value the guidance of our Tropical HTIRC advisory committee (see *listing below*) in helping to prioritize our strategic directions, and we have added several new members: Tomas Höök (Purdue), Natalie Kurashima (Kamehameha Schools), Kylle Roy (USDA

Forest Service), and Michael Streshley (Terraformation).

In 2025, we also completed a project in collaboration with DOFAW to conduct a statewide assessment of forestry nurseries operated or managed by the State of Hawai'i, with a vision toward potentially expanding production capacity. Our results (*summarized in this annual report*), suggest a strong increase in future demand for seedlings to help accomplish landscape level native forest restoration in Hawai'i. An important outcome from this project was that DOFAW created



19-year-old planted koa at Pu'uwa'awa'a.

a new “Statewide Horticulture Coordinator” position, which we expect to be filled in 2026.

In February 2025, we held our Tropical HTIRC annual advisory committee meeting at Terraformation’s Ridge-to-Reef Restoration Center (3RC) in Kailua-Kona, providing an opportunity to tour their nursery and seed bank on site. During our field day, we visited Terraformation’s ‘Ōhi’a Lani at Waikoekoe restoration site near Waimea and Kaiāulu Pu’uwa’awa’a, a community-based subsistence forest area at the Pu’uwa’awa’a Forest Reserve within the Hawai’i Experimental Tropical Forest. We are grateful to our partners, Terraformation and Akaka Foundation for Tropical Forests, for hosting us!

In this 2025 Annual Report, we feature accomplishments from 10 of our ongoing projects designed to help meet our research, extension, and education strategic objectives. These projects continue to be led mainly by our graduate students at Purdue University and University of Hawai’i at Mānoa, in direct collaboration with Tropical HTIRC partners.

Mahalo for your collaborations and commitment to Hawai’i forests.

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**Douglass F. Jacobs**

Director, Tropical HTIRC

## Tropical HTIRC Advisory / Steering Committee

The role of the committee is to provide guidance on the strategic directions of the center, prioritization of research themes, and staffing. The committee is comprised of members representing diverse groups associated with ecology and management of Hawai'i's forests. Many of the committee members are active collaborators on Tropical HTIRC research and extension projects.

Member	Title, Institution
<b>Steve Bergfeld</b>	Hawai'i Island Branch Manager, Hawai'i DLNR Division of Forestry and Wildlife
<b>Daniel Bowman</b>	State Forester, USDA Natural Resources Conservation Service, Pacific Islands Area
<b>Kuali'i Camara</b>	'Āina Mauna Land Manager, Department of Hawaiian Home Lands
<b>Susan Cordell</b>	Research Ecologist, Institute of Pacific Islands Forestry, USDA Forest Service, Pacific Southwest Research Station
<b>Nicklos Dudley</b>	Forestry Team Leader and Maunawili Experiment Station Manager, Hawai'i Agriculture Research Center
<b>J.B. Friday</b>	Extension Forester, University of Hawai'i at Mānoa
<b>Christian Giardina</b>	Forest Ecologist, Institute of Pacific Islands Forestry, USDA Forest Service
<b>Matt Ginzel</b>	HTIRC Director, Entomology and Forestry and Natural Resources, Purdue University
<b>Jennifer Grimm</b>	Forest Management Specialist, Hawai'i DLNR Division of Forestry and Wildlife
<b>Neil Hannahs</b>	Siglo Tonewoods
<b>Robert Hauff</b>	State Protection Forester, Hawai'i DLNR Division of Forestry and Wildlife
<b>Tomas Höök</b>	Department Head, Forestry and Natural Resources, Purdue University
<b>Greg Hendrickson</b>	General Manager, Kealakekua Mountain Reserve
<b>Travis Idol</b>	Professor of Tropical Forestry and Agroforestry, University of Hawai'i at Mānoa
<b>Zachary Judd</b>	Forestry Manager, Parker Ranch
<b>Natalie Kurashima</b>	Integrated Resource Manager, Kamehameha Schools
<b>Nicholas Koch</b>	Siglo Tonewoods
<b>Creighton M. Litton</b>	Professor of Ecosystem Ecology, University of Hawai'i at Mānoa
<b>Robert K. Masuda</b>	Senior Advisor, Forestry and Natural Resources, Purdue University
<b>Kylle Roy</b>	Forest Entomologist, Institute of Pacific Islands Forestry, USDA Forest Service
<b>Irene Sprecher</b>	Forest Solutions, Inc.

## Tropical HTIRC Staff



**Erin Bell** is a PhD student with the Department of Forestry and Natural Resources at Purdue University. She earned her undergraduate degree in Biology from Carroll College in 2014 and her master's degree from Miami University in 2020. Prior to enrollment at Purdue, she gained experience in ecological research in Idaho and Montana with the USDA Forest Service before moving to work in the diverse ecosystems of Hawai'i. Working with Maui Forest Bird Recovery Project and their partners since 2019, she developed a passion for native plants and the unique relationships they hold with Hawai'i's forest birds. Erin is conducting analyses of

restoration areas on Hawai'i Island as well as exploring the role of native avian seed dispersers in these sites to influence future management decisions for restoring avian habitat.



**Solomon Champion** completed his PhD in the Botany Department at the University of Hawai'i at Mānoa during 2025, studying the genetics of native Hawaiian sandalwood tree species. Solomon's interests are in genomics, phylogenetics, and evolution. He used population genomic methods to elucidate population structure within the Hawaiian sandalwoods and identified hybrid individuals compromising the Hawaiian endemic sandalwoods. Solomon is now working for DLNR / DOFAW: Snail Extinction Prevention Program, analyzing metagenomic data from Hawaiian land snail host plants and culturing microbes to better understand their ecology in Hawaiian forests.



**Kelly French** is a PhD candidate at Purdue University, Department of Forestry and Natural Resources. Kelly earned her BA in Biology from Colgate University in 2015 and worked for four years in human genetics while spending most weekends working on her family's 450-acre Tree Farm in Maine. This drove her to return to school to study forestry, and in 2021 she graduated with her MS in Forest Resources from the University of Maine where she studied tree ecophysiology. During her PhD, Kelly is investigating 'iliahi ecology and physiology, determining optimal conditions for 'iliahi outplantings in the hope of informing future restoration

management strategies. Through her fieldwork at Parker Ranch and greenhouse trials at Purdue University, she is assessing how microenvironment, microclimate, and host species influence 'iliahi performance and water/carbon relations.



**Jennipher Himmelmann** is a Resource Assistant with the USDA Forest Service Urban and Community Forestry Program and recent addition to the Tropical HTIRC staff. She is based on Hawai'i Island where she earned her undergraduate degree in Environmental Science from the University of Hawai'i at Hilo and began her career in forestry as an intern. During her time at UH Hilo, she worked in the Listening Observatory for Hawaiian Ecosystems identifying forest bird vocalizations to train machine learning algorithms, and completed a Senior Thesis under the Spatial Data Analysis and Visualization Lab. Jennipher loves the opportunity to apply her

skills in GIS and communications to provide administrative and technical support to partners in tropical forestry conservation in the place she calls home.



**Asa McCurdy** is a PhD candidate with the Department of Forestry and Natural Resources at Purdue University. He earned his undergraduate degree in Plant Sciences and Technology from the University of Missouri-Columbia in 2020 and his master's degree in Botany and Plant Pathology from Purdue University in 2023. Asa's PhD project focuses on the link between nonstructural carbohydrate (NSC) and chemical defense dynamics of 'ōhi'a trees to predict their resistance to Rapid 'Ōhi'a Death, a destructive fungal pathogen complex. Asa is collecting samples from mature 'ōhi'a trees to quantify NSC reserves and chemical defenses throughout

the year and using this foundational knowledge to inform subsequent field and greenhouse trials. While Asa's thesis will focus on NSC dynamics, collaboration with members of the Trowbridge Lab at the University of Wisconsin-Madison will elucidate defense compound dynamics. Asa aims to use these NSC and chemical defense compound data to inform decision making for ROD management and mitigation strategies.



**Tawn Speetjens** is a PhD student at Purdue University in the Department of Forestry and Natural Resources (FNR). He was born and raised in Honoka‘a on the Big Island of Hawai‘i, and graduated from the University of Hawai‘i at Hilo in 2009 with a BS in Biology, and from Purdue University in December 2022 with a MS from the Department of Forestry and Natural Resources. He has worked throughout diverse ecosystems in Hawai‘i with highlights that include working for the US FWS on Laysan Island in the northwestern Hawaiian Islands, banding forest birds at the Hakalau National Wildlife refuge, and conducting watershed management

with the Kohala Watershed Partnership. He also worked as a forester and nursery manager for the Hāloa ‘Āina Reforestation Project where he was introduced to the unique challenges of growing Hawaiian sandalwood species (‘iliahi). Tawn has a passion for growing native plants of Hawai‘i and is interested in ecological restoration and commercial production of ‘iliahi and native Hawaiian natural resources. He led development of a *S. paniculatum* propagation protocol that was published in *Tree Planters Notes* in 2021. His MS research examined the effects of fertilizers and host pairing on nursery production and field establishment of *S. paniculatum* and he published the first chapter of his thesis in the journal *Forests* in 2023. Tawn continues to work with *S. paniculatum* in his PhD research as he explores the physiological mechanisms that underlie the ‘iliahi-host connection.



**Pandu Wirabuana** is a PhD graduate (2025) from the Department of Natural Resources and Environmental Management at the University of Hawai‘i at Mānoa. He received his BS and MS from Universitas Gadjah Mada, Indonesia. His dissertation focused on mixed stand management between ‘iliahi and host plants in Hawaiian tropical dry forests, specifically addressing the balance of competition, facilitation, and parasitism between ‘iliahi-host at the stand level. His dissertation committee was comprised of Drs. Travis Idol (supervisor), Douglass Jacobs, J.B. Friday, Michael Saunders, and Kasey Barton.



Tropical HTIRC

**STRATEGIC DIRECTIONS**

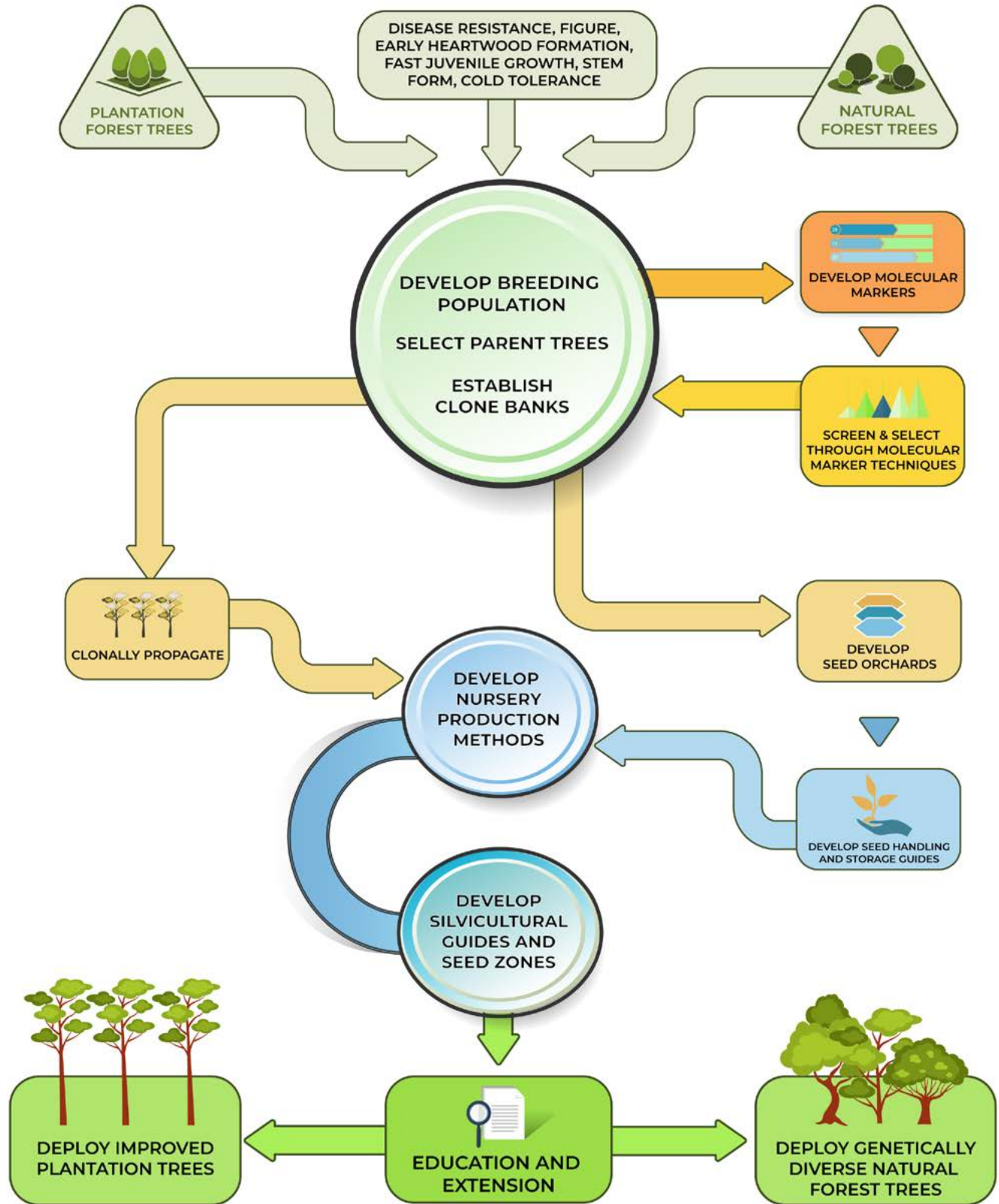


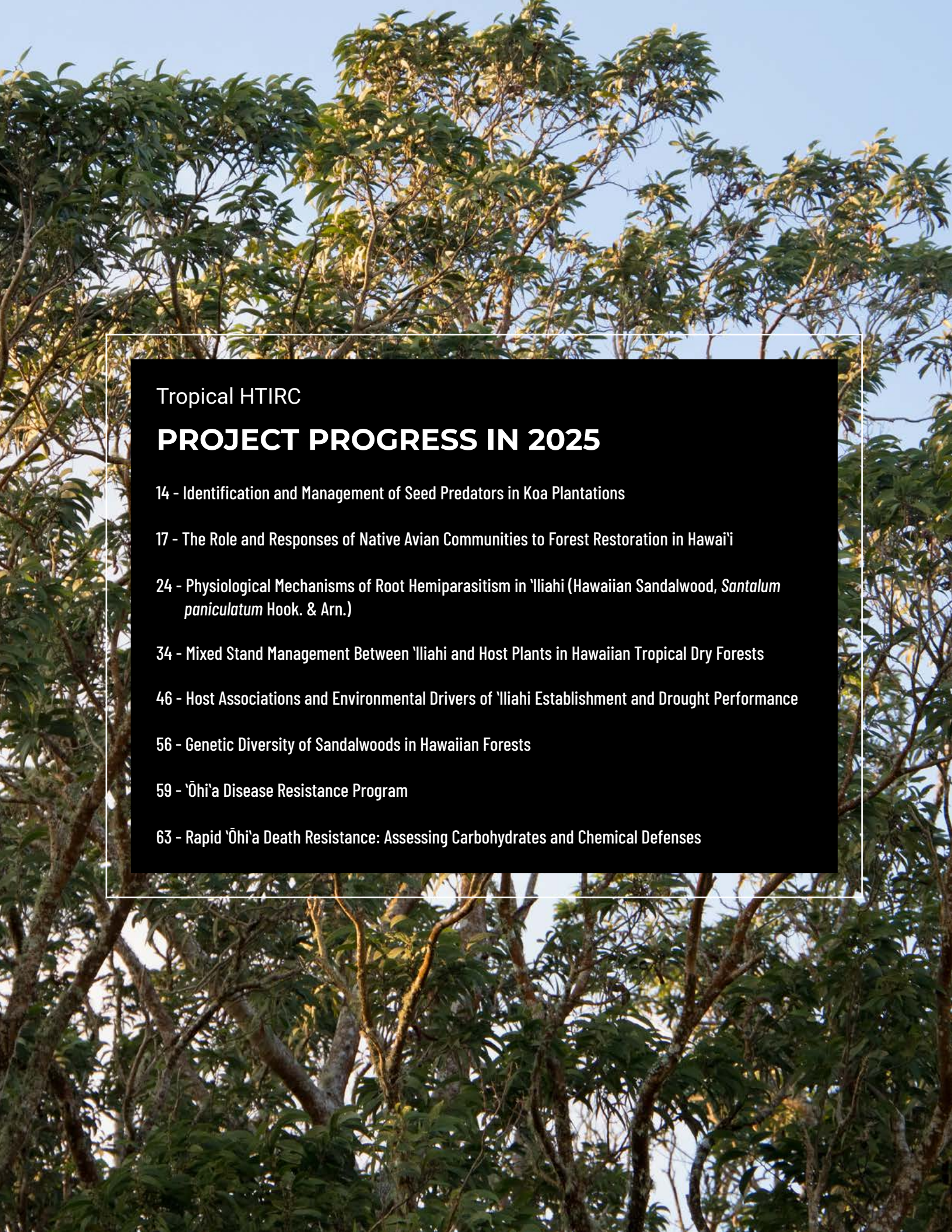
## Strategic Directions

1. Improve the genetic quality of tropical hardwoods, especially *Acacia koa*, through use and refinement of traditional tree improvement methods and novel molecular marker techniques.
2. Develop advanced nursery propagation and seed production technologies to improve the field establishment success of tropical hardwood reforestation / restoration and allow for efficient production of genetically improved trees.
3. Establish a system of native species research and demonstration trials (e.g., on the Hawai'i Experimental Tropical Forest) for education and training of consultant and industrial foresters, nursery practitioners, conservation biologists, and public and private landowners in silviculture and regeneration techniques.
4. Educate future leaders in tree improvement, management, and protection of tropical hardwood forests.
5. Engage our stakeholders and address their needs by communicating research findings and management recommendations.

# Tropical HTIRC Program Structure / Road Map

We aim to address these themes in an interconnected way for native Hawai'i forest trees including koa, 'ōhi'a, and 'Iliahi.





Tropical HTIRC

## **PROJECT PROGRESS IN 2025**

14 - Identification and Management of Seed Predators in Koa Plantations

17 - The Role and Responses of Native Avian Communities to Forest Restoration in Hawai'i

24 - Physiological Mechanisms of Root Hemiparasitism in 'Iliahi (Hawaiian Sandalwood, *Santalum paniculatum* Hook. & Arn.)

34 - Mixed Stand Management Between 'Iliahi and Host Plants in Hawaiian Tropical Dry Forests

46 - Host Associations and Environmental Drivers of 'Iliahi Establishment and Drought Performance

56 - Genetic Diversity of Sandalwoods in Hawaiian Forests

59 - 'Ōhi'a Disease Resistance Program

63 - Rapid 'Ōhi'a Death Resistance: Assessing Carbohydrates and Chemical Defenses

# Identification and Management of Seed Predators in Koa Plantations

## INVESTIGATORS

**Kylle Roy:** Forest Entomologist, USDA Forest Service, Forest Health Protection, Pacific Southwest Region

**Ellen Dunkle:** Forest Entomology Associate, Pacific Cooperative Studies Unit, University of Hawai'i at Mānoa

**Dan Mikros:** Entomology Associate, University of Hawai'i at Hilo

**Angelita Acebes-Doria:** Research Biologist, USDA-ARS, Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center

**Pascal Aigbedion-Atalor:** Assistant Professor, University of Hawai'i at Mānoa, Department of Plant and Environmental Protection Sciences

**Kyhl Austin:** PhD Candidate, University of Hawai'i at Mānoa, Department of Plant and Environmental Protection Sciences

**Aileen Yeh:** Consultant, Hawai'i Agriculture Research Center

**Kevin Burke:** Consultant, Siglo Tonewoods

**Nick Koch:** General Manager, Siglo Tonewoods

**Nicklos Dudley:** Senior Forest Scientist, Hawai'i Agriculture Research Center

## BACKGROUND / JUSTIFICATION

Koa wilt, caused by *Fusarium oxysporum* f. sp. *koae*, has decimated populations of koa (*Acacia koa*), a bioculturally and economically significant tree endemic to Hawai'i. Over a decade of genetic breeding has resulted in the identification of wilt resistant seed,

however, several insect pests are known to predate on the seed, damaging up to 90% of seed yield. This issue has not been investigated in 40 years; therefore, the current seed predators were previously unknown. In addition, pest control methods to improve wilt resistant seed yield had not been implemented prior to this study.

## PROJECT OBJECTIVES

Determine the koa seed predators in both wilt resistant koa stands and natural koa populations across the State of Hawai'i

Test various seed predator control techniques ultimately to develop an integrated pest management plan. To date, we have tested:

- Aerial spray of Delegate WG (a.i. 25% spinetoram)
- Mating disruption using Isomate OFM TT

## APPROACH

Using citizen science, we have presented our project ideas at multiple local meetings including Hawai'i Conservation Conference, Pacific Entomology and Botany Meeting, and Trop HTIRC, and requested koa seed pods to rear out potential seed predators. We reared insects from brown and green koa seed pods in 2024 and 2025.

We conducted two trials at Agricultural Research Center at Maunawili, O'ahu in the Spring of 2024 and 2025. Here, a 1-acre stand of wilt-resistant koa trees was treated



Koa seed worms on *Cryptophlebia illepida* consuming seeds of green koa seed pods.



Aerial deployment of Delegate WG at Maunawili, O'ahu, HI.

with Delegate WG via a drone aerial sprayer and we compared moth trap capture and reared seed predators to a untreated 1-acre wilt resistant koa stand.

At Siglo Tonewoods on Hawai'i Island, we tested Isomate OFM TT in the Spring of 2024 and 2025. We compared seed predators reared from koa seed pods and moth trap capture from a 3-acre plot of wilt resistant koa treated with Isomate OFM TT to a second, slightly larger untreated stand.

### KEY FINDINGS

We received samples from over 10 sites and data has revealed the major seed predators as *Cryptophlebia illepada*, or koa seedworm. As of 2025, seed predation ranged from 4%-98%.

Both Isomate OFM TT and Delegate WG appear to have reduced seed predation at Maunawili and Siglo Tonewoods.

### FUTURE PLANS

We are continuing all three projects in 2026; both Isomate OFM TT and Delegate WG will need Special Local Needs labels to be used in the State of Hawai'i.

Please submit koa seed pods to: [kylle.roy@usda.gov](mailto:kylle.roy@usda.gov)

- At least 50 seedpods all brown or all green
- Preferably 5 pods from a single tree (10 trees)
- Record site name, GPS Coordinates, and elevation

### PARTNERS AND COLLABORATORS

USDA Forest Service

State of Hawai'i Division of Forestry and Wildlife

University of Hawai'i at Mānoa, College of Tropical Agriculture and Human Resources, Cooperative Extension

Siglo Tonewoods

Hawai'i Agricultural Research Center

Oahu Army Natural Resources Program

Mauna Kea Watershed Alliance

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# The role and responses of native avian communities to forest restoration in Hawai'i

## INVESTIGATORS

**Erin Bell**, PhD Graduate Student, Department of Forestry and Natural Resources, Purdue University

**Michala Philips** (Committee member), Research Ecologist, Pacific Island Ecosystems Research Center, US Geological Survey

**Pat Zollner** (Committee member), Professor, Department of Forestry and Natural Resources, Purdue University

**John Dunning** (Co-advisor), Professor, Department of Forestry and Natural Resources, Purdue University

**Douglass Jacobs** (Co-advisor), Fred M. van Eck Professor, Department of Forestry and Natural Resources, Purdue University

## BACKGROUND / JUSTIFICATION

In Hawai'i, restoring native habitats is fundamental for conserving the endemic species and maintaining the ecological integrity of the region. The proposed projects aim to address key aspects of habitat conservation and restoration, focusing on the impact of restoration efforts on avian communities, the role of birds in seed dispersal and forest regeneration, and the effectiveness of translocating frugivores to restore ecological functions.

By evaluating the outcomes of restoration projects on bird populations, the first project will provide insights into the effectiveness of different restoration areas and their

implications for biodiversity conservation. The second project seeks to understand the mechanisms of seed dispersal by birds and the factors influencing forest recovery that will inform the development of targeted restoration approaches that enhance ecosystem resilience and biodiversity. The third project looks at assessing the dispersal patterns and habitat utilization of translocated frugivores, contributing to our understanding of their role in ecosystem restoration and the re-establishment of essential ecological functions.

## PROJECT OBJECTIVES

### Objective 1: Impact of Restoration Projects on Avian Communities

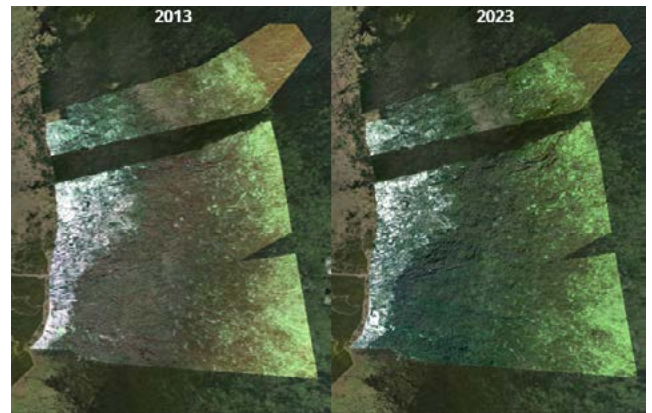
Small-scale restoration efforts can support native birds, but the response of bird populations to restoration varies depending on several factors. This project analyzes the impact of restoration projects on avian populations in selected sites on Hawai'i Island, considering species-specific responses and canopy cover change. We employ remote sensing and supervised classification to evaluate changes in vegetation structure at each restoration site. The two sites being analyzed are Hakalau Forest National Wildlife Refuge and Pu'uwa'awa'a Forest Reserve on Hawai'i Island. Each site has undergone years of differing restoration efforts, resulting in diverse changes within the canopy structure and grass cover. After classification and quantification of the vegetation changes

in these areas, analyses from past forest bird surveys will then be run to determine the differences in avian communities within these areas over the past ten years. By understanding the specific responses of different bird species to canopy change, this objective aims to provide insights that can guide more efficient and effective forest restoration strategies for wildlife habitat, ensuring the long-term survival of endemic birds and the overall health of the ecosystem.

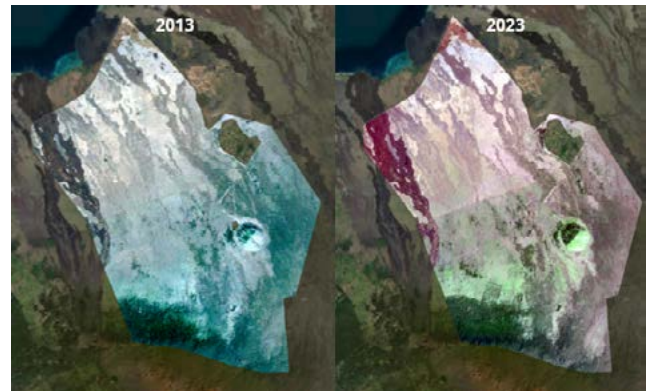
### Objective 1 Approach:

#### *Area Classification and Remote Sensing Analysis*

- High-resolution satellite imagery for Hakalau NWR and Pu'uwa'awa'a FR from 2012-2022 will be acquired (Figure 1a and b).
- The imagery will be corrected for atmospheric conditions, sensor errors, and other distortions, normalizing brightness and contrast across all images.
- Indices like the Normalized Difference Vegetation Index (NDVI) will be utilized to assess vegetation density and type.
- Supervised classification techniques will be trained to differentiate between canopy cover, grass cover, impervious surfaces, and water (Figure 1c).
- Changes in area and density of each category will be calculated to assess vegetation change each year.
- The supervised classification will be tested and calibrated for accuracy, utilizing random ground truthing points.
- Change detection algorithms will



**Figure 1: a) Hakalau Forest National Wildlife Refuge restoration area 2013 vs 2023, raw imagery.**



**Figure 1: b) Pu'uwa'awa'a Forest Reserve restoration area 2013 vs 2023, raw imagery.**

be employed to identify significant alterations in vegetation over time.

- Significant and non-significant changes will be reported and maps created to display landscape alterations.

#### *Point Count Analysis*

- Data from the Hawai'i Forest Bird Interagency Database Project will be utilized from each of the transects that exist within the restoration areas. This data follows point-transect sampling procedures to record detection type and distance to detected birds.
- Distance sampling methods will be used

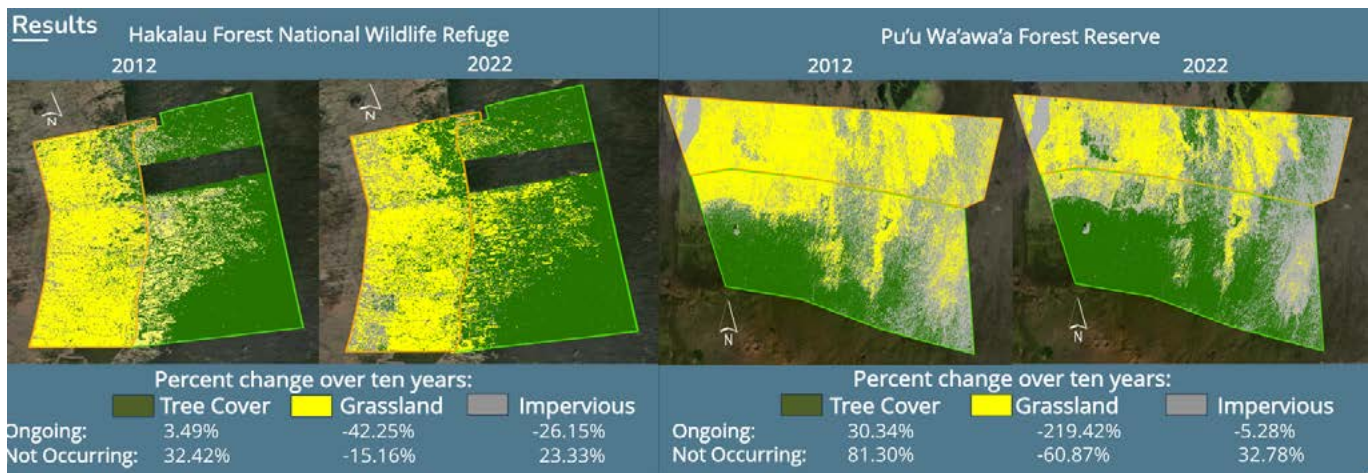


Figure 1: c) Classified imagery between the two sites and percent change over ten years where restoration is ongoing (area in light green) and not currently occurring (orange).

to correct for undetected individuals and estimate bird densities.

- Analysis on the relative abundance of selected bird species in restoration areas over a 10-year period within the areas displaying change in canopy cover and vegetation will occur.
- Trends in the bird populations in areas with and without forest restoration will be compared.

**Objective 2: Informing Management Decisions for Avian Habitat Restoration Utilizing Seed Dispersal**

Restoration ecologists face challenges in re-establishing native populations and ecosystem processes in degraded environments. Effective and cost-efficient methods for habitat restoration in Hawai'i are still being explored, with varying results across different sites. Seed dispersal by birds can be helpful for forest recovery, especially in areas adjacent to intact forests or forest fragments. This project seeks to identify key information that can inform management decisions aimed at restoring avian habitat

adjacent to intact forests or forest fragments. We will investigate the role of birds in seed dispersal and the factors that influence the recovery of tropical forests, particularly in former agricultural lands. By setting up transects of seed rain traps outside of intact forest, we will provide comprehensive data on the furthest extent of bird-mediated seed dispersal and its potential impact on habitat restoration. This study aims to examine seed rain travel and the effects of avian dispersal, contributing to effective management decisions that can be tailored to the specific ecological and environmental conditions of the Hawaiian Islands.

**Objective 2 Approach:**

*Seed Dispersal Distance, Measurements, and Distribution*

- We will measure seed rain in Hilo Forest Reserve and Pu'uwa'awa'a FR by placing hoop style traps along transects within these areas. These transects begin within the intact forest and extend into the open matrix (pasture/grassland). Traps are at least 20 meters apart from one another

and will be placed under remnant trees within the open matrix or under perches (in the absence of remnant trees). There are 10 transects per site, and each transect extends at least 200 meters into the open matrix (Figure 2a and b).

- Trap contents will be collected once a month from March 2025 to April 2026, sorted, and seeds will be identified and recorded (Figure 2c).

#### *Fruit Abundance*

- All fruiting plant species within a 20m radius of each trap will be recorded during each seed rain collection visit. All fruiting plant species along the forest edge will also be recorded during this time. This will help inform which seeds we would expect to find in the traps.
- Measures of fruiting activity for each species surveyed will be calculated, including percent occurrence and indexed abundance.
- Patterns in seed rain and fruit phenology will be compared using daily seed rain averages.

#### *Germination Trials*

- Seeds collected from the traps that are determined to be avian-dispersed will be planted in similar conditions (in a greenhouse setting) to seeds collected from trees in the same areas.
- Rates of germination and growth will be measured until seedlings are identifiable and compared.

### **Objective 3: Dispersal and Habitat Utilization by Translocated Frugivores**

The abundance of mobile organisms like birds is closely related to resource availability,

and their movements are essential for services like seed dispersal and pollination. Translocation of native frugivores, such as the 'alalā (*Corvus hawaiiensis*) and 'ōma'ō (*Myadestes obscurus*), has been proposed as a management tool to restore seed dispersal functions in areas where native frugivores are extinct (see former 'ōma'ō range in Figure 3a). The success of bird translocations depends on factors such as survival rates, dispersal behavior, and long-term population persistence. This project focuses on understanding how a translocated frugivore population, specifically the 'ōma'ō, disperses and utilizes restored habitats. We aim to assess the role of these translocated frugivores in restoring seed dispersal functions in forests where native frugivores no longer exist. By examining the survival rates, dispersal behavior, and habitat utilization of translocated 'ōma'ō, we seek to evaluate the effectiveness of reintroduction as a conservation strategy. The research will contribute to understanding the ecological impacts of translocated frugivores on plant community composition and seed dispersal services, providing valuable insights for future conservation efforts aimed at restoring and preserving Hawai'i's unique avian biodiversity and ecosystems.

### **Objective 3 Approach**

- Beginning September 2025, we captured 28 birds and translocated 20 'ōma'ō individuals from stable populations in Windward Hawai'i to the Leeward Pu'uwa'awa'a Forest Bird Sanctuary (Figure 3b).
- Birds were captured using standard mist-netting procedures and processed for banding and VHF radio transmitter fitting.



Figure 2a - Layout of the seed rain trap transects at Pu'uwa'awa'a Forest Reserve.

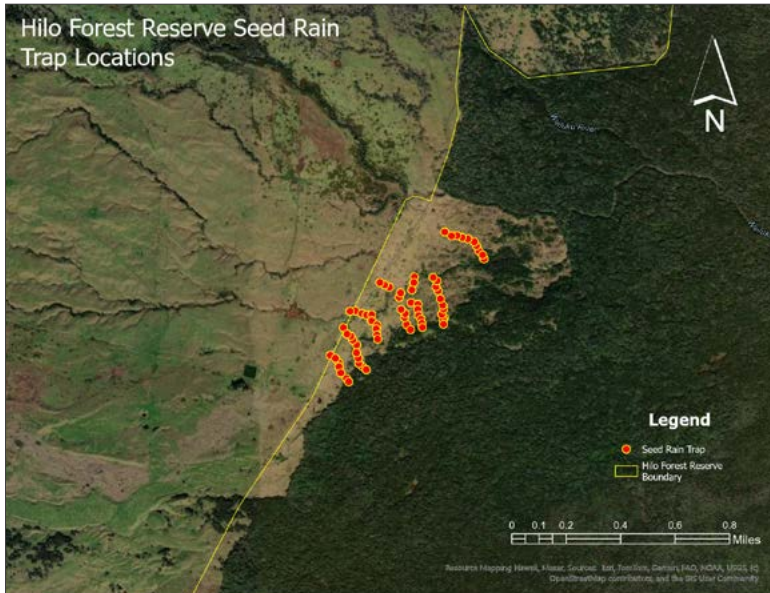


Figure 2b - Layout of the seed rain trap transects at Hilo Forest Reserve.

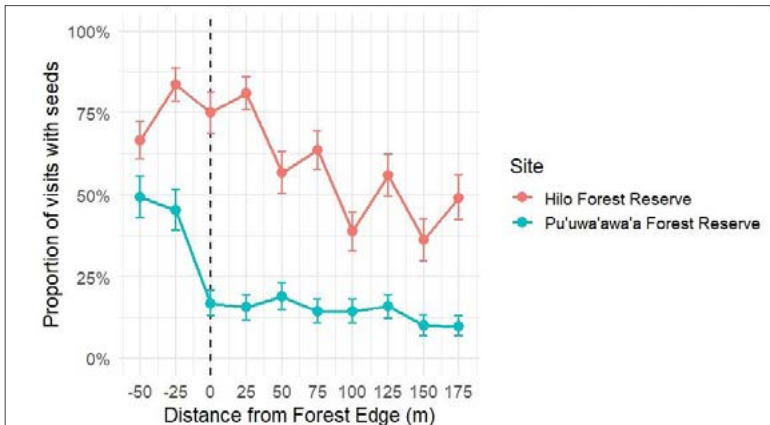


Figure 2c - Preliminary results showing frequency of seed presence (dispersal mechanism not identified) by distance from forest edge at HFR and PWW.

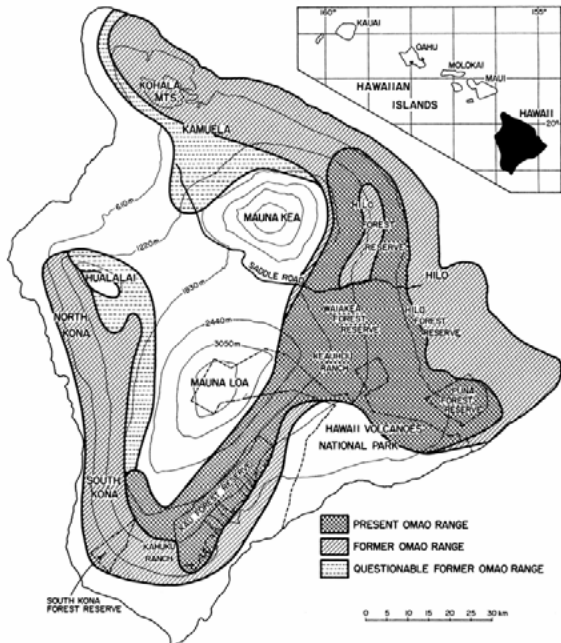


FIGURE 1. Map of Hawaii showing former, questionable former, and present day range of *Phacoria obscurus*.

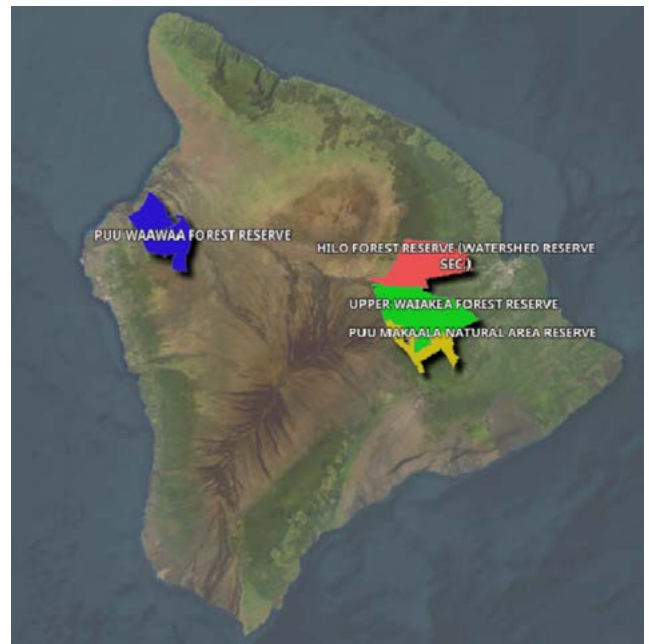


Figure 3: b) Windward collection sites in blue shades, Leeward release site in yellow.

Figure 3: a) Former range of the 'ōma'ō from Van Riper and Scott 1979.

- Captured individuals were transported and released at Pu'uwa'awa'a FR. We tracked their dispersal and movements, habitat preferences, and interactions using VHF telemetry.
- Movement patterns, home range size, and behaviors were analyzed to understand their adaptation to the new environment. Home range maps were created and diet composition will be determined from the field observations (Figures 3c and d).
- 17 individuals remain on the landscape to date, long-distance dispersal is limited and most birds have accumulated in the Southwest corner of the Forest Bird Sanctuary where understory growth is abundant. Birds have been observed

- foraging on native plant species and utilizing forest overstory and understory.
- The next phase of captures and movement to supplement the current translocated population will commence in late spring/early summer 2026 after breeding season has finished.
- After the transmitters are dropped or run out of battery life, annual surveys to estimate the population of released birds will be conducted.

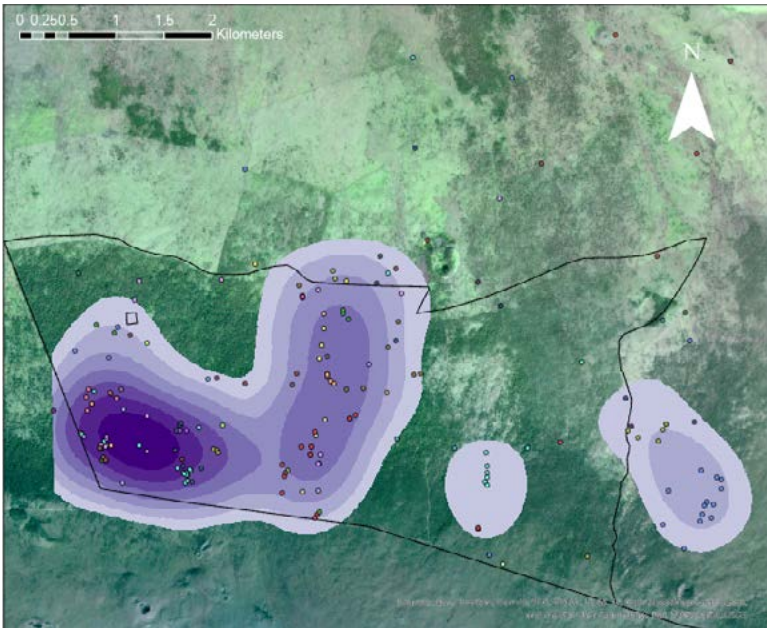


Figure 3: c) Core use area for total translocated 'ōma'ō population.

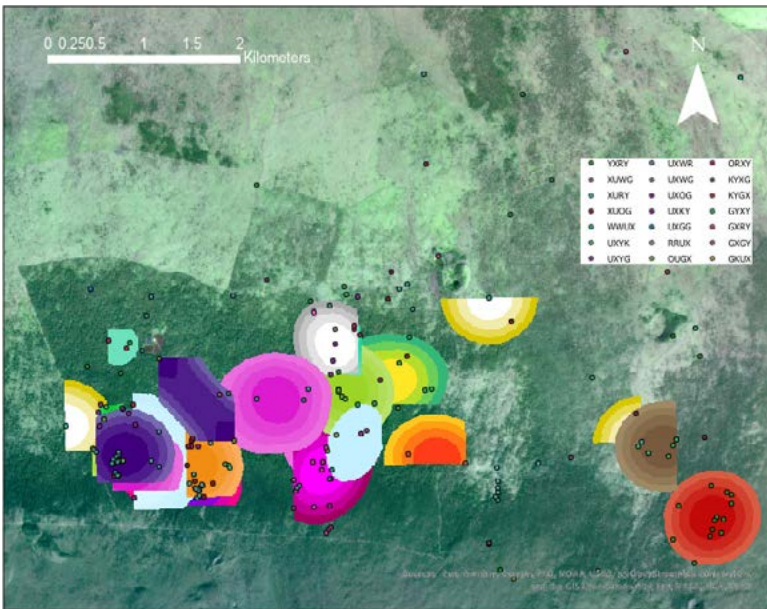


Figure 3: d) Core use area for each released individual.

### PARTNERS / COLLABORATORS

Hawai'i Division of Forestry and Wildlife- Alex Wang and supporting staff

Research Corporation of the University of Hawai'i- Cara Thow and supporting staff

United States Geological Survey- Rick Camp

# Physiological Mechanisms of Root Hemiparasitism in 'Iliahi (Hawaiian Sandalwood, *Santalum paniculatum* Hook. & Arn.)

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## BACKGROUND / JUSTIFICATION

There is significant interest in cultivating 'iliahi for both ecological restoration and commercial production. The selection of suitable host species may facilitate the growth of hemiparasitic plants, yet literature addressing host selection for 'iliahi is limited. Furthermore, the physiological mechanisms underlying root hemiparasitism in *Santalum* are poorly understood, further complicating the prospect of effective host selection. Developing a better understanding of these

mechanisms of root hemiparasitism may be pivotal in selecting suitable host species.

Root hemiparasites, such as those in the genus *Santalum*, must first contact their hosts' roots to initiate haustoria connections. However, it is not fully understood how the roots of *Santalum* species find their hosts' roots. A recent study with *S. album* demonstrated that root exudates from the high-quality host *Dalbergia odorifera* can stimulate *S. album* root growth and may contain haustoria-inducing factors that act as chemical signals to guide *S. album* root growth towards host roots and to stimulate haustorium formation (Li et al., 2023). *S. paniculatum* forms well-developed haustoria with several Hawaiian native species, including *Acacia koa* and *Metrosideros polymorpha*. However, it is not known whether 'iliahi responds to host-borne chemical signals like *S. album*. Additionally, *S. paniculatum* haustoria have been observed attached to various surfaces besides plant roots, including N-fixing nodules of *A. koa* and inorganic surfaces such as controlled-release fertilizer beads, cinder, and perlite, further complicating the understanding of the physiological triggers driving haustoria development.

Haustoria are specialized parasitic nodules that serve as the site of resource transfer from the host to 'iliahi and effectively create

a persistent interspecies root graft between 'iliahi and a broad range of host species. Typically, interspecific tissue grafts in plants do not persist in distantly related species due to defense responses deteriorating the connection. The ability of *Santalum* species to maintain interspecific root grafts suggests that the successful and lasting establishment of haustoria on host roots depends on the manipulation of both the host's and the *Santalum* species' defense responses. The association of *S. album* with another suitable host, *Cajanus cajan*s, was shown to alter the biochemical defense responses in both parasite and host (Mohapatra & Anil, 2022). However, the candidate genes underlying this response have not been identified.

Once haustoria connections are established, it is believed that the gradient of plant water potential between 'iliahi and their hosts drives the transfer of resources, and

this is supported by observations of leaf water potential of *Santalum* species being consistently lower than their connected hosts (Světlíková et al., 2018), although this has not been explicitly demonstrated in a controlled setting. Resource transfer occurs through indirect xylem connectivity, where xylem-borne resources are predominantly transferred from the host to 'iliahi, although the transfer of resources in the opposite direction has also been detected in *S. album* with its hosts in the field plantation setting (Lu et al., 2020). Additionally, the transfer of resources from *Santalum* to its host may expose the host to *Santalum*-borne chemical signals that help reduce the host's defense response. Transfer of sRNA from a parasitic plant to its host has been shown to reduce the defense response of the host in other parasitic plants in the genus *Cuscuta* (Johnson & Axtell, 2019)



**Figure 1:** Preliminary trials completed with the X-ray CT scanner showed detectable root growth within a 1-month interval for *S. paniculatum*.

The objective of this project is to examine the physiological mechanisms of root hemiparasitism in *S. paniculatum* to develop a greater understanding of the parasite-host relationship. We will conduct three experiments to examine the different mechanisms underlying root hemiparasitism across three phases of parasitic association. First, we will focus on the development of *S. paniculatum* roots as they grow to encounter their host, using X-ray Computed Tomography (CT) to nondestructively monitor root architecture and haustorium development during parasitic association. Second, we will use RNA-seq to assess differential gene expression associated with plant defense responses in *S. paniculatum* and its host in response to parasitic association. Third, we will use stable isotope tracers to monitor resource transfer between *S. paniculatum* and a host as we manipulate the water potential gradient between them. This work will ultimately help to improve our understanding of the complex dynamics of root hemiparasitism and better inform the host selection process for 'iliahi and other *Santalum* species beyond Hawai'i.

## EXPERIMENT 1

### Root architecture and haustoria development of the root hemiparasite (*Santalum paniculatum*) in response to host pairing and haustoria-inducing factor (HIF) 2-6-dimethoxy-p-benzoquinone (DMBQ).

- Obj. 1: Use X-ray CT imaging to nondestructively monitor root architecture development in response to association with an *Acacia koa* host.
- Obj. 2: Determine whether the application

of DMBQ can enhance the growth and haustoria connections in native host species pairings.

- Obj. 3: Assess the efficacy of using X-ray CT imaging to detect haustoria connections.

### Experimental Design

This experiment will use nondestructive X-ray CT imaging to monitor root development and RGB and hyperspectral imaging to monitor shoot development of *S. paniculatum* in response to association with an *A. koa* host and to applications of the potent haustoria-inducing factor DMBQ. DMBQ enhances root growth and haustoria establishment in *S. album*, and if the same response is observed in *S. paniculatum*, application of DMBQ may serve as a valuable cultural practice to improve cultivation of *S. paniculatum*.

We will use a two-factor design with a full factorial combination of host association (2 levels: hosted vs control) and DMBQ application (2 levels: applied vs control), yielding 4 unique treatments. We will pair 24 *S. paniculatum* seedlings with an *A. koa* host, and 24 *S. paniculatum* seedlings will remain unpaired as a control, for a total of 48 sample containers. Twelve samples from each *S. paniculatum*-host pair and twelve control *S. paniculatum* (unpaired) will receive a solution of dilute DMBQ, and the remaining half of the samples will not receive any DMBQ. Samples will be grown together for 8 months in a completely randomized design (CRD) and rearranged monthly. We will use the Purdue Alumni Seed Phenotyping Facility (AAPF) to complete root scans with an X-ray

CT scanner (Fig. 1) and shoot scans with RGB and hyperspectral imagery. We will conduct root-and-shoot scans immediately after planting and then at bimonthly intervals throughout the 8-month growing period, producing 6 points in a time series.

### Assessment

X-ray CT technology generates a high-resolution 3D digital twin of the scanned root systems, enabling nondestructive measurement of root architecture and subsequent extraction of morphological traits. Morphological traits extracted from the digital twins will include root volume, root mass, root surface area, root length, distribution of root mass along a depth gradient, and mean branching angle.

We have conducted preliminary experiments that show that X-ray CT can also detect haustoria connections forming with the host. Therefore, we will also utilize X-ray CT to monitor characteristics of haustorium development, including the number of haustorium connections, the diameter of targeted host roots, and the soil depth at which attachments occur. We will destructively sample all plants at the end of the grow-out period to assess root morphology on a flatbed scanner, count haustoria, and measure attached host roots, and compare these measurements with data generated from X-ray CT phenotyping.

We will use RGB imaging to generate 3D digital representations of the shoot to assess morphological characteristics, including shoot height, leaf number, leaf area, and ground-level diameter. We will also use

hyperspectral imaging to assess several indices shown to indicate water stress, which may become apparent upon the establishment of haustorium connections.

### Updates

We conducted a limited trial to determine the optimal soil moisture content that maximizes the contrast between growing media and roots and improves imaging results. The system requires soil to be dried down to a minimum of 20% field capacity to maximize contrast without causing embolism due to water scarcity. We also conducted a trial to determine the perlite-to-potting soil ratio that allows the soil to dry down to 20% holding capacity within 30-35 days of being hydrated to 40% field capacity, following scans. We found that a ratio of 3 parts perlite to 1 part potting soil dried down at a rate sufficient to achieve this scanning interval.

Seeds of *S. paniculatum* are currently germinating and will initially be transplanted into 105 mL root-training containers, where they will grow for 2 months prior to planting in pots used for root and shoot scans. Once seedlings have grown sufficiently so that their roots fill the 105 mL container, they will be transplanted into 3-gallon pots with hosts, where they will grow for the duration of the scanning period. Root and shoot scans are scheduled to begin in June 2026.

## EXPERIMENT 2

**Influence of parasitic association on gene expression and biochemical defense responses of root hemiparasite (*Santalum paniculatum*) and host (*Cajanus cajan*s).**

- Obj. 1: Explore general changes in gene expression of *S. paniculatum* and a *C. cajans* host in response to pairing.
- Obj. 2: Determine whether genes associated with the synthesis and transport of defense response signaling hormones jasmonic acid (JA) and salicylic acid (SA) are differentially expressed by *S. paniculatum* or the host in response to pairing.
- Obj. 3: Assess changes in plant biochemical defenses in *S. paniculatum* and the *C. cajans* host in response to pairing.

### Experimental Design

The experiment will be a two-factor design with *S. paniculatum* (2 levels: present vs absent) and Host: *C. cajans* (2 levels: present vs absent) as the predictor factors, combined in an incomplete factorial to form 3 treatments. For the RNA-seq analysis, mRNA will be extracted from three tissue types (1. Haustoria, 2. Fine root, 3. Leaf) for *S. paniculatum*, and two types (1. Fine root & 2. Leaf) for *C. cajans*. Comparison of gene counts to identify differentially expressed genes will be performed between similar tissue types from paired and unpaired *S. paniculatum* and from paired and unpaired hosts. Mean gene counts will be calculated across five biological replicates, and each biological replicate will be composed of tissue pooled from 5 individuals. Thirty total cDNA libraries will be sequenced for *S. paniculatum* (5 biological reps. x 3 tissue types x 2 (paired vs unpaired)), and 20 for *C. cajans* (5 biological reps x 2 tissue types x 2 (paired vs unpaired)). We will grow 25 parasite-host pairs, 25 unpaired *S. paniculatum*, and 25 unpaired *C. cajans*.

Paired and unpaired host seedlings will be transplanted into designated containers when the *S. paniculatum* is 6 months old. The parasite-host pair, along with unpaired *S. paniculatum* and unpaired hosts, will be grown for 12 additional months to allow sufficient time for the parasitic association to form.

### Assessment

We will use the HiSeq 2000 platform to generate 100 bp paired-end reads from cDNA libraries generated from the extracted mRNA. Sequence reads for *S. paniculatum* will be aligned to a *S. album* reference genome (Zhang et al., 2023), and *C. cajans* will be aligned with a *C. cajans* reference genome (Varshney et al., 2012) to generate mean gene counts. We will generate three lists of differentially expressed genes from the comparison of haustoria, root, and shoot tissue between paired and unpaired *S. paniculatum*. We will then generate two lists of differentially expressed genes from the comparison of root and shoot tissue between paired and unpaired *C. cajans*.

We will analyze the list of differentially expressed genes to determine whether genes associated with jasmonic acid and salicylic acid biosynthesis and signaling are influenced by parasitic association in the various tissues sampled.

The concentration of jasmonic acid and salicylic acid in three tissue types will be measured using LC-ESI-MS/MS at the metabolite profiling facility at Bindley Science Center at Purdue University. We will measure the activity of superoxide dismutase (SOD) and guaiacol peroxidase (POX), and quantify nonstructural carbohydrates and soluble sugars, total phenols, and total flavonoids

in each tissue type. We will incorporate all tissue we collect for the genetic and biochemical defense analyses into the dry mass calculations to assess biomass accumulation in response to predictors as well. Nondestructive measurements of plant morphology (height, ground line diameter, number of leaves, chlorophyll content) will occur monthly throughout the 12-month growing period.

### Updates

We currently have 50 *S. paniculatum* seedlings, one month old, growing in the Horticulture and Landscape Architecture (HLA) greenhouse facility at Purdue University. *C. cajans* seedlings will be germinated and transplanted in 3.5-gallon pots with the *S. paniculatum* in July 2026 when the *S. paniculatum* are 6 months old. Destructive sampling, RNA extraction, and sequencing will occur in July of 2027.

## EXPERIMENT 3

### Influence of 'iliahi-host plant water potential gradient on the rate and direction of haustoria-borne resource transfer.

Obj. 1: Determine the influence of the water potential gradient between the 'iliahi and its host on the degree and direction of resource transfer.

Obj. 2: Determine if concurrent resource transfer occurs through haustoria connections.

### Experimental Design

We will use a single-factor design in which we vary which plant in an *S. paniculatum*-*A. koa* pairing is manipulated to increase its water potential. We will manipulate the water potential of the target plant by enveloping

it in a clear plastic bag while pumping fog into the bag to create a supersaturated atmosphere, thereby reducing the vapor pressure deficit and leaf transpiration of the bagged plant. There will be three levels of the water potential manipulation treatment; first, neither plant will be bagged to assess the flow of resources under unadulterated conditions (control), then we will bag the *A. koa*, which should increase the degree of the parasite host water potential gradient, and lastly we will bag the *S. paniculatum* to reduce the parasite host water potential gradient.

Pairs will be grown together for 12 months to ensure they are connected, then the tracer experiment will occur over 8 days. We will apply a  $^{15}\text{N}/^{13}\text{C}$ -labeled urea solution to the leaves of an *A. koa* host and destructively sample both plants in each pair at 2-day intervals over 8 days as the bagging treatment is applied.

### Assessment

Real-time water plant water potential will be recorded during the 8-day experiment using a passive camera monitoring system (Cavicam) that captures micro variations in stem swelling as a proxy for water potential (Fig 2). The Cavicam system works by establishing a baseline area of a leaf petiole at maximum hydration, then comparing reductions in petiole area to that maximum as leaf water potential declines (Fig 3). The real-time water potential assessment will allow monitoring of diurnal fluctuations in the water potential gradient throughout the experiment.

Destructive sampling will occur at 0, 2, 4, 6, and 8 days. Destructive sampling of the root, stem, and leaf tissue from both plants in the



Figure 2: Cavacam set up requires cutting a window of the leaf to create a silhouette of the leaf midrib to detect changes in the leaf midrib area.

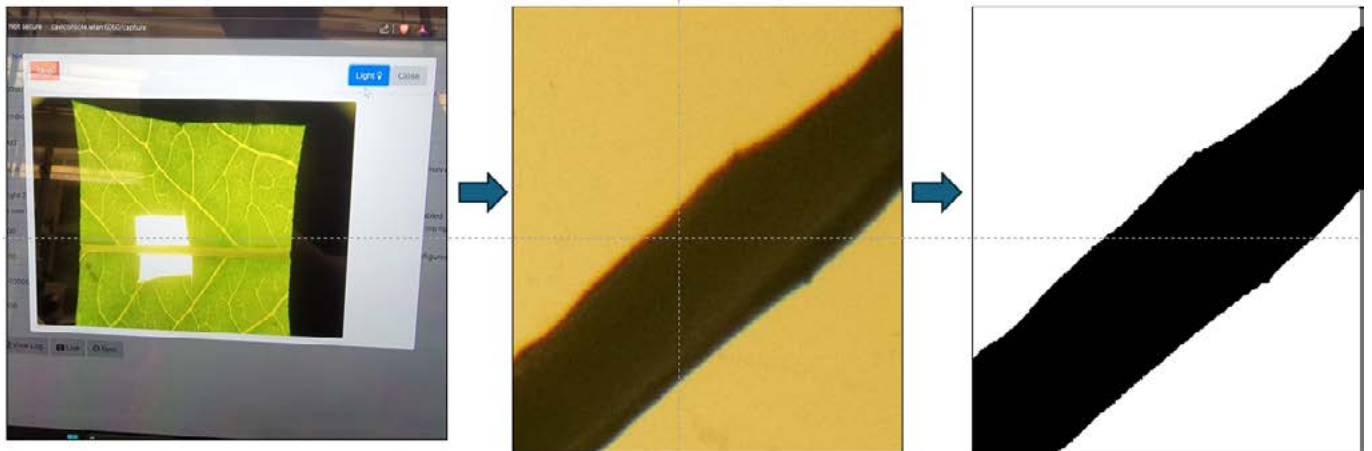


Figure 3: Images of the leaf midrib are binarized and the pixels representing the leaf midrib are counted to detect microvariations in midrib size as leaf water potential changes.

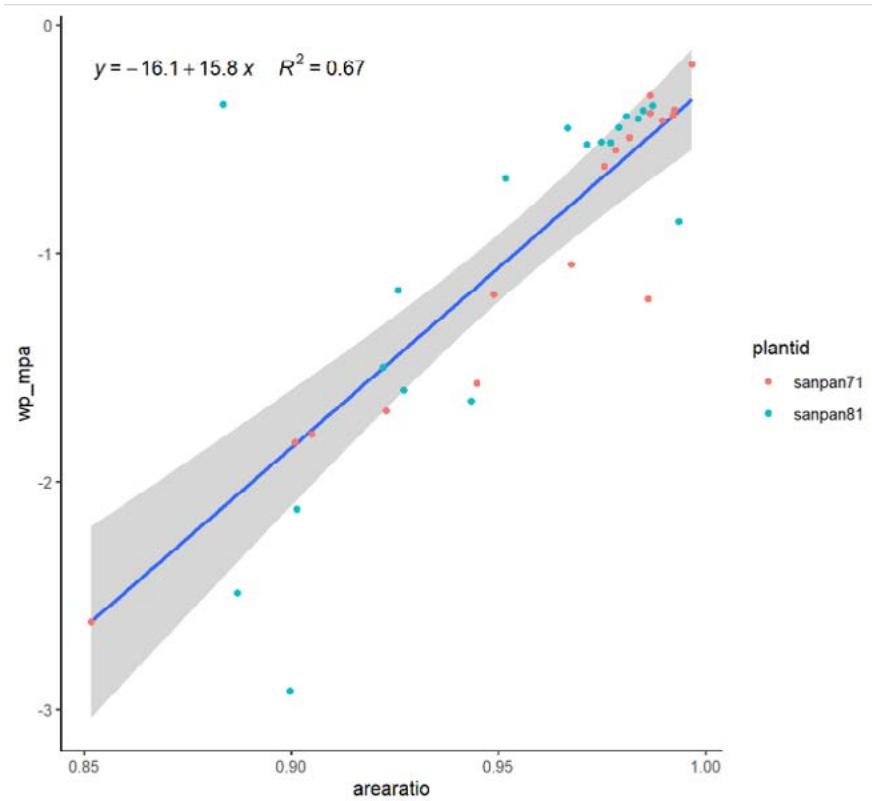


Figure 4: Plotting the relationship between the ratio of leaf midrib area to the maximum leaf midrib area to leaf water potential shows a fairly strong relationship between the two, allowing us to use this method to conduct real time water potential sampling.

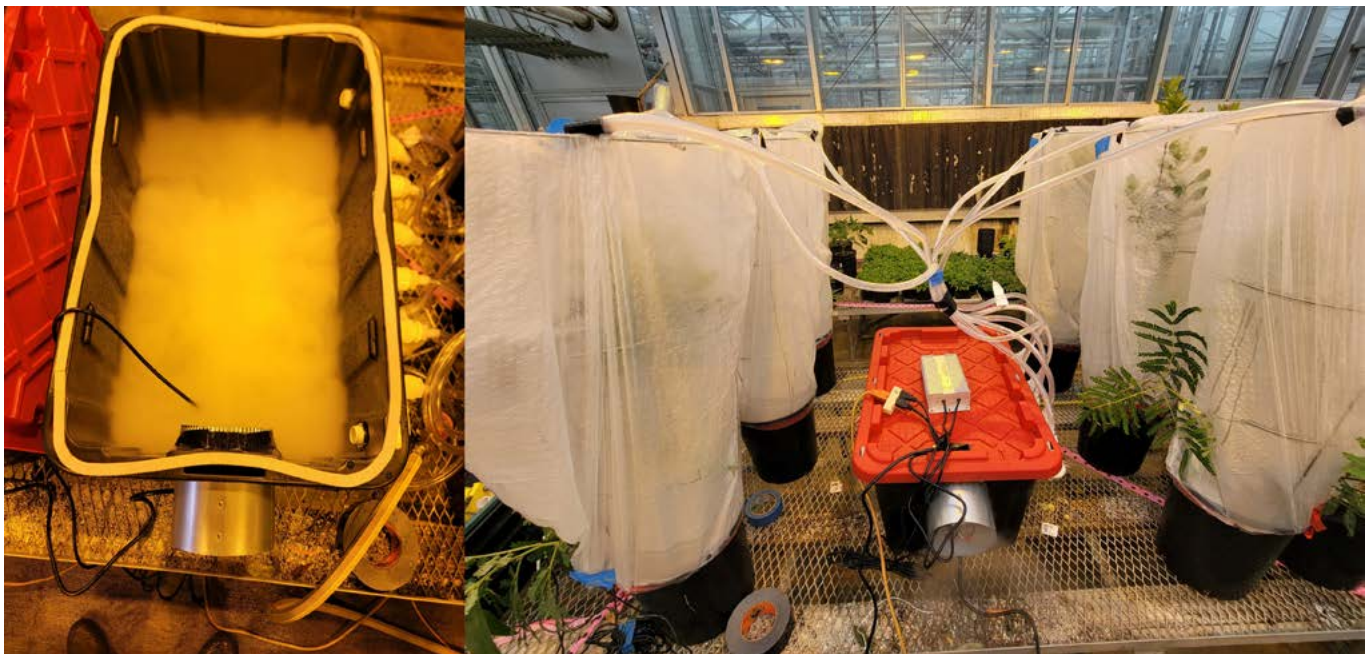


Figure 5: A preliminary trial of the bagging and fogging system provided proof of concept to support the use of this method to manipulate water potential of the bagged plant.

container will be performed at each time point. The levels of  $^{15}\text{N}$  and  $^{13}\text{C}$  enrichment in each tissue type will be measured using an elemental analyzer–isotope ratio mass spectrometer (EA-IRMS). We will compare the levels of  $^{15}\text{N}$  and  $^{13}\text{C}$  enrichment in each tissue type for *S. paniculatum* and the host across each bagging scenario to determine the effect of manipulating the water potential gradient on the degree of resource transfer over time.

### Updates

We have worked with the McAdam lab at Purdue University to construct 24 new Cavicam systems to collect real-time water potential measurements throughout the experiment (Fig 2). To use the Cavicam effectively to monitor water potential, we must ensure that the relationship between leaf water potential and petiole area is consistent across individuals. *S. paniculatum* petioles are not long enough to reach the field of view of the cameras used in the cavicam system, so we were required to conduct a trial that would determine the feasibility of using the leaf midrib in place of the petiole, as well as to ensure that the relationship between leaf water potential and leaf midrib area was consistent across several *S. paniculatum* individuals (Fig 3). Our results showed that the relationship between leaf water potential and midrib area was consistent across three individuals (Fig. 4).

We designed and constructed a fog production and distribution system using a 53-gallon storage tote, an inline duct fan, and an industrial pond fog generator (Fig 5). Preliminary trials testing the effectiveness of the bagging treatment showed that the

water potential of bagged *S. paniculatum* increased effectively, reducing the water potential gradient measured at three time points (11:30, 14:00, 16:40) (Fig 6). The tracer experiment is scheduled to take place in June 2026.

### FUTURE PLANS

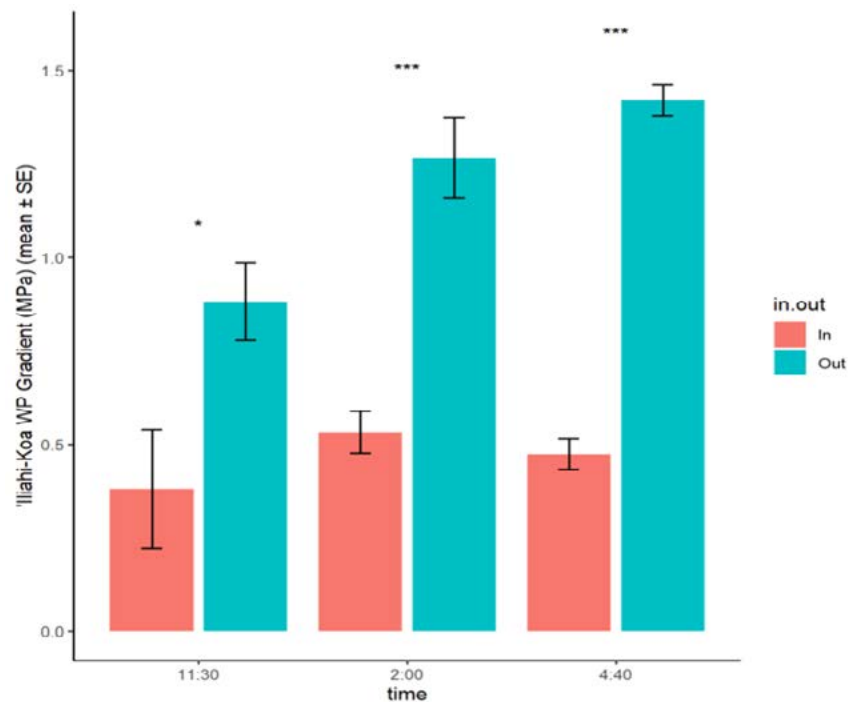
Seedlings for the X-ray CT experiment are currently germinating and will be transplanted into containers for scanning in June 2026. *S. paniculatum*-*A. koa* pairs grown for the tracer experiment are nearing 9 months old and the tracer experiment will take place in June 2026. The 'iliahi seedlings for the RNA-seq experiment are currently 1-month old and the sampling for RNA extraction and sequencing will occur in July 2027.

### PARTNERS AND COLLABORATORS

The Hāloa 'Āina reforestation project has provided all the plant material for the described experiments.

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**Figure 6:** Comparison of the *S. paniculatum*-host water potential gradient of pairs with *S. paniculatum* bagged (In) vs pairs with unbagged *S. paniculatum* (Out) showed significant differences in the gradient at three time separate time points (11:30, 14:00, and 16:40)

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# Mixed Stand Management Between 'Iliahi and Host Plants in Hawaiian Tropical Dry Forests

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## BACKGROUND / JUSTIFICATION

Recovering populations of sandalwood ('iliahi) is a priority for biodiversity conservation in Hawaiian tropical dry forests. Two previous field studies on Hawai'i Island have been initiated to explore planting designs for reforestation of *S. paniculatum* ('iliahi) and host species. In the first study, 'iliahi seedlings were underplanted in a 10-yr-old koa plantation. Seedling survival was >95% and average seedling height and diameter growth were 40 cm and 4.3 mm,

respectively in the first year after planting (Thyroff et al. 2022). Seedling photosynthesis and growth after three years were positively related to canopy openness (Thyroff et al. 2024). 'Iliahi seems healthier with better growth when it is surrounded by more hosts (Figure 1). This suggests parasitism and competition were both occurring and strongly affecting performance of 'iliahi. It also suggests that pre-establishing a host plant can improve both 'iliahi seedling performance and the continued survival and vigor of the host.

Another study is related to the host suitability evaluation wherein 'iliahi seedlings were outplanted simultaneously with one of two native woody host species: *Dodonaea viscosa* ('a'ali'i) and *Acacia koa* (koa) (Thyroff et al., 2023). Establishing 'iliahi closer to koa provided better survival, height, and basal stem diameter growth. Moreover, field observation after initial growth periods suggested three possibilities of comparative growth between sandalwood and the host: (a) both host and 'iliahi had good growth; (b) the host exhibited better growth than 'iliahi; and (c) 'iliahi showed better growth than the host (Figure 2). This suggests that the host does not always facilitate 'iliahi establishment; there may also be a significant competitive interaction that is not fully mitigated by parasitism. Results from this early field study suggest a high level of variation among 'iliahi-host seedling pairs in parasitic resource transfer and relative growth during the first one to three years.

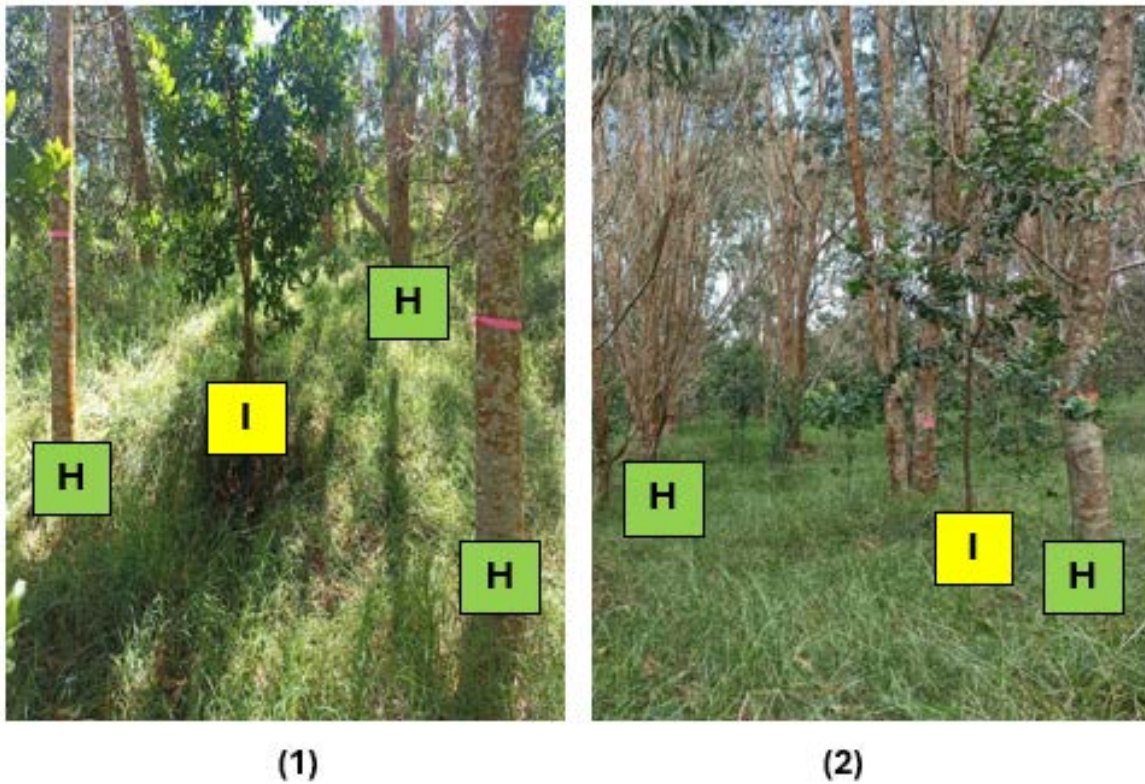


Figure 1. Growth of ‘iliahi saplings in underplanting experiment: (1) ‘iliahi is healthier and growth is better when surrounded by more hosts; (2) Greater canopy openness results in better ‘iliahi growth. This picture was taken using a mobile camera on August 2024.

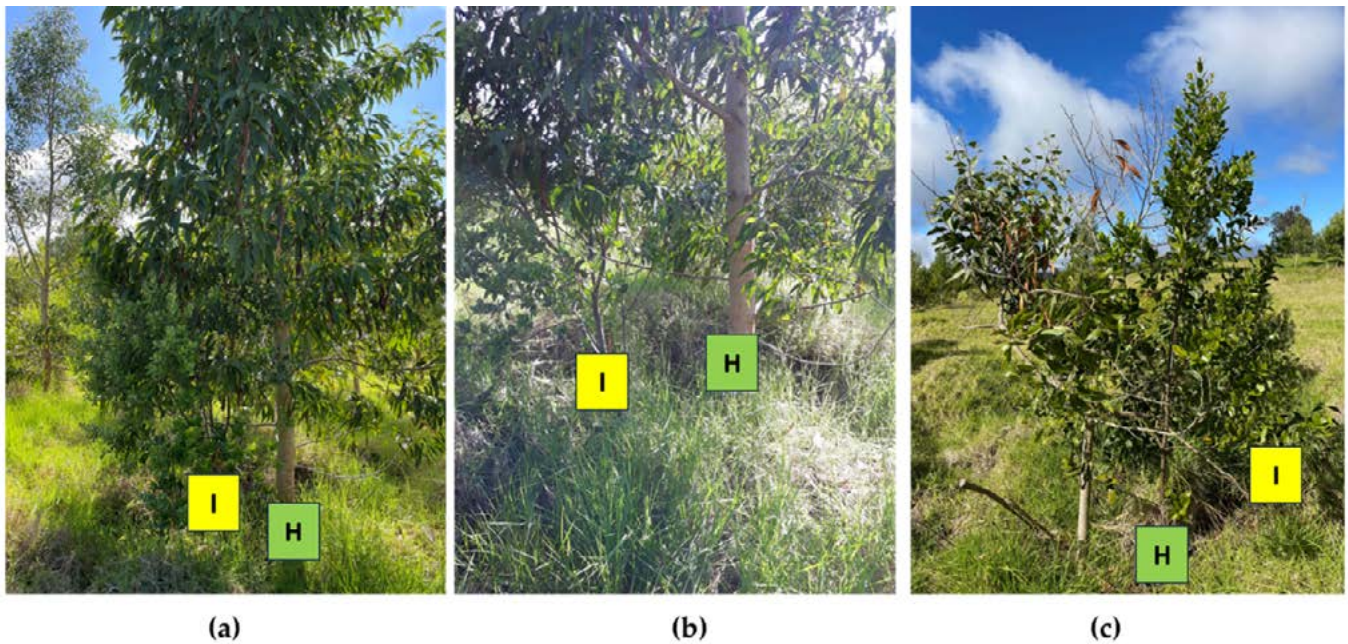


Figure 2. Comparison of growth between ‘iliahi and host in the site experiment of a reforestation project in January 2023. The trial was established in 2020, wherein host and ‘iliahi were planted simultaneously. Three possibilities for performance were captured, i.e. (a) both ‘iliahi and host had good growth; (b) host exhibited better growth than ‘iliahi; and (c) ‘iliahi demonstrated better growth than host.

Although the host plays an important role as a resource provider for 'iliahi, competition between the host and 'iliahi also occurs, and these two interactions may influence each other. There may be a threshold of host density or proximity to ensure 'iliahi seedling establishment, and host species may have a threshold tolerance to root parasitism. The limiting factors that affect these interactions are still unknown and may vary by host species. Multiple hosts are likely required to support long-term survival and growth of 'iliahi, but the suitable or optimal species composition and spacing are unknown. Further investigation is required to address several questions, including:

1. What is the balance competition between 'iliahi and host plants in controlling facilitation and parasitism among them and the associated impact on the growth of 'iliahi and the host?
2. How do plant species composition and host type abundance affect this balance and plant growth?
3. Can limiting factors be identified that influence the interaction between host and 'iliahi and their impact on growth?

## PROJECT OBJECTIVES

- Estimate the relative strengths of competition, facilitation, and parasitism among 'iliahi and host plants
- Quantify the optimum 'iliahi-host plant ratio in balancing competition, facilitation, and parasitism among 'iliahi and hosts
- Measure differences in resource availability in different combinations of plant basal area, species composition,

and 'iliahi: host ratios as potentially limiting factors for the growth of 'iliahi and host plants.

## APPROACH

### Plant Competition

The first project aims to estimate the balance of competition, facilitation, parasitism among underplanted 'iliahi saplings and overstory koa trees. Shading by overstory koa trees will limit growth of 'iliahi as well as parasitic resource transfer of water and nutrients from koa since 'iliahi has a range of threshold tolerance to shade level. We divided this site into a grid of 20×20 m plots (Figure 3). It was designed to obtain a forest community structure from the combination of surviving 'iliahi and koa. Plots naturally varied in the number and size of 'iliahi and koa due to the variation of survival and growth for both species. Stem diameter at breast height (DK) were used as parameters to assess the growth of koa as the host plant. Growth of 'iliahi was measured based on ground-line stem diameter (DS) and plant height (HS). Plot-basal area was assumed as the indicator of competition at the stand level, while facilitation and parasitism were estimated using mixed-species growth models based on the relative growth of each species across different 'iliahi-host plant ratio.

### 'Iliahi-Host Ratio

The second project aims to quantify the optimum 'iliahi-host plant ratio in balancing competition, facilitation, and parasitism among 'iliahi and host plants in mixed stands. We evaluated the influence of stand composition and host abundance on 'iliahi and host growth. As with the underplanting

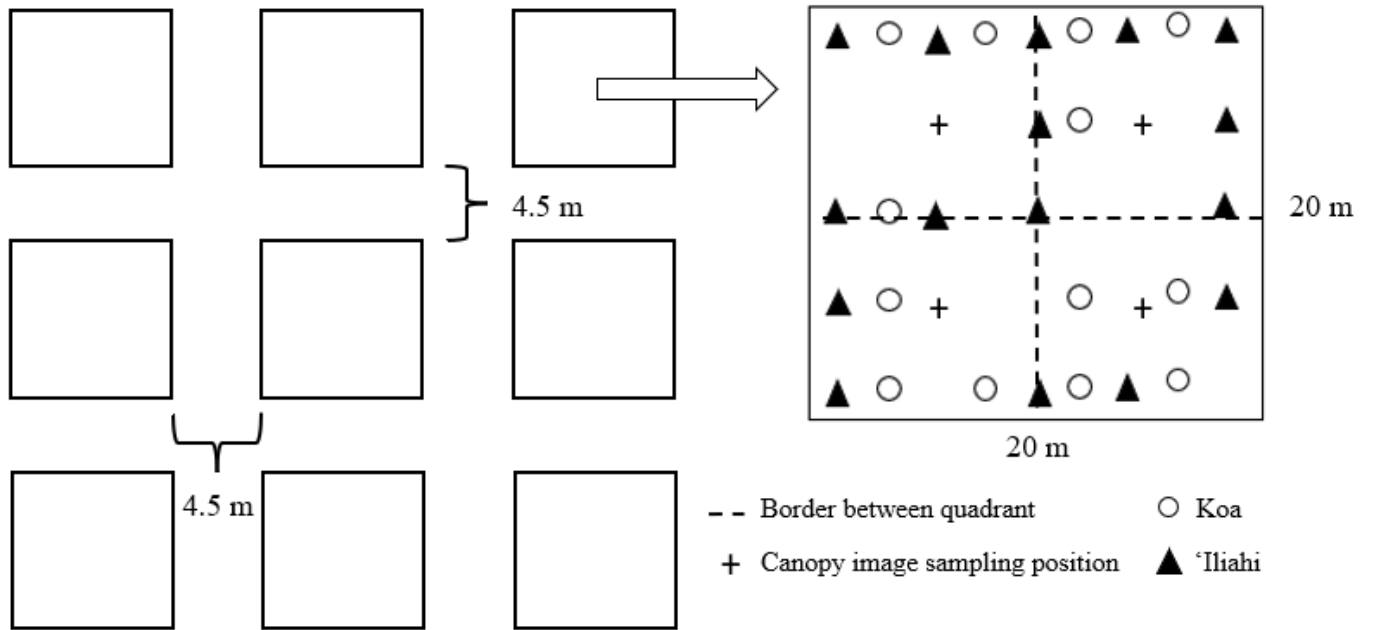


Figure 3. The sketch of plot-based area and plant position inside each plot in the koa underplanting site. The layout is developed based on the preliminary observation on January 4, 2023 and trial measurement on March 15, 2023, assuming that all plants inside the plot survive.

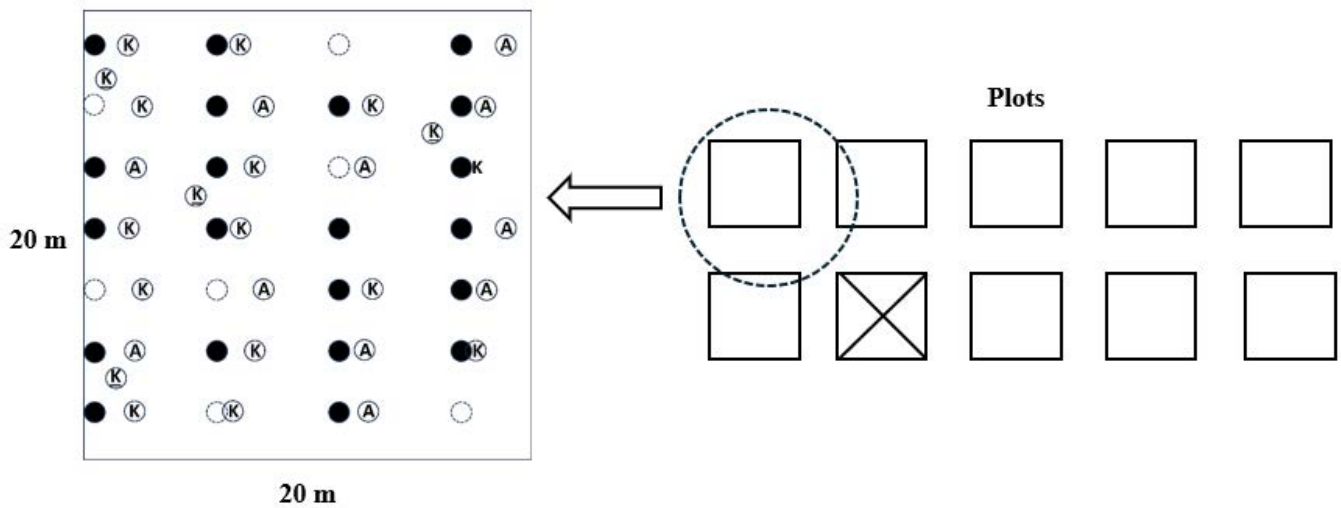
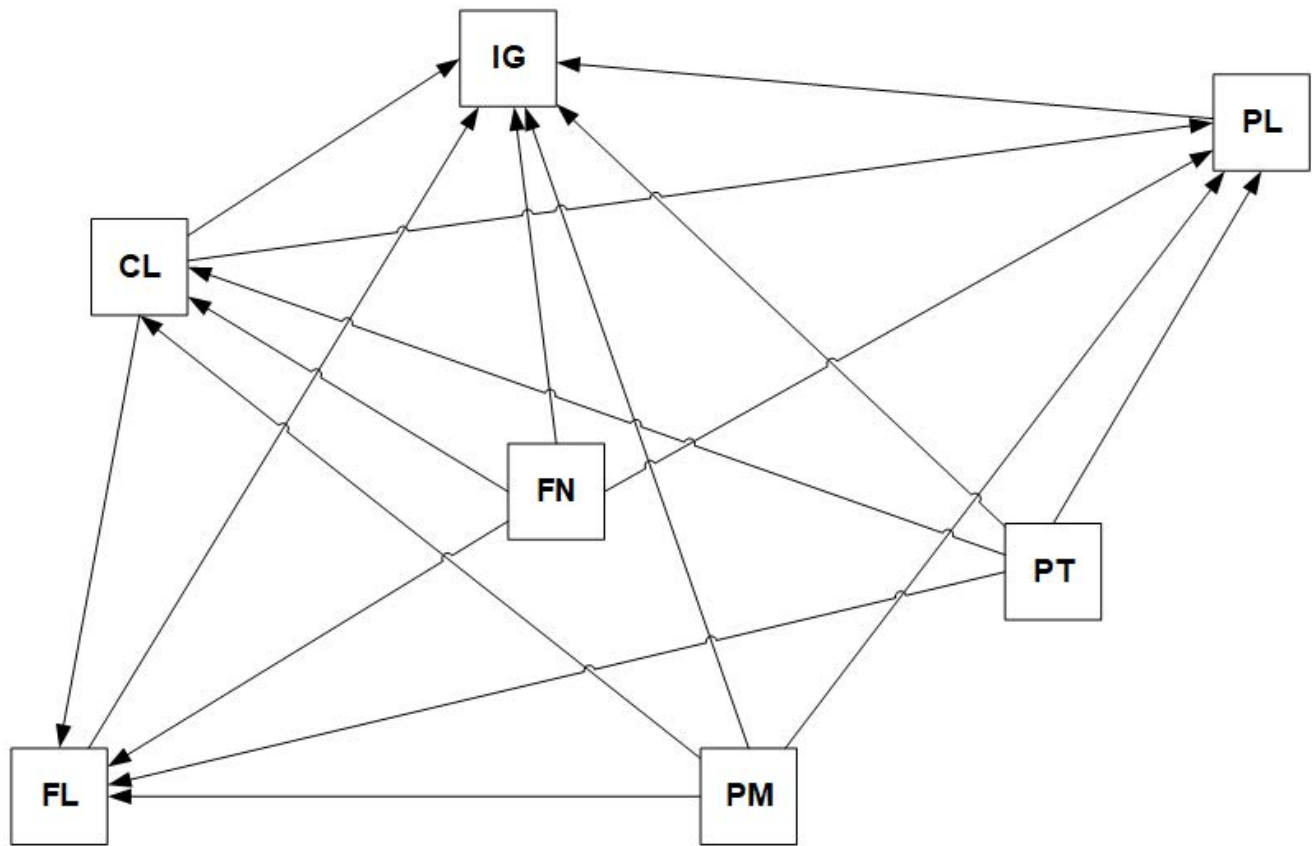


Figure 4. Sketch of a 20 x 20 m plot, including borders of adjacent plots, based on the original host suitability experimental design. Filled circle, surviving 'Iliahi; dotted unfilled circle, dead 'Iliahi; A, 'a'ali'i planted as host; K, koa planted as host; K, koa naturally regenerated



**Figure 5. The concept of structural equation modeling for determining limiting factors on ‘iliahi growth in Hawaiian tropical dry forests.** Description: PT (planting time), PM (planting mixture), FN (proportion of N<sub>2</sub>-fixing to non-fixing host), CL (competition), FL (facilitation), PL (parasitism), IG (‘iliahi growth). The arrow indicates a directional effect of variables.

experiment, we expected that 5 years after outplanting, the saplings in this site were large enough to be interacting across experimental units within and between rows. This provided an opportunity to investigate the plant interactions at a stand levels in plot-based experimental units rather than ‘iliahi-host pairs. There has been mortality of some plants, which differs by treatment (Thyroff et al. 2023), as well as natural regeneration of koa and seedlings of other woody plants. To address these larger scale interactions, we split the experimental site into adjacent 20×20 m plots that each contain four of the original ‘iliahi-host experimental units

within four columns and seven rows (Figure 4). Plant composition varied among plots depending on the combination of previous treatments, plant mortality, and plant regeneration. Ground-line diameter (*d*) and plant height (*h*) were used to evaluate the growth of all woody plants inside plot. For koa saplings with a height >3 m, diameter was measured at breast height (~1.3 m). Foliar nutrients concentration was also recorded for ‘iliahi only.

### Factors Limiting ‘Iliahi Growth

The third project aims to identify factors limiting planted ‘iliahi growth in Hawaiian tropical dry forests. It addressed the

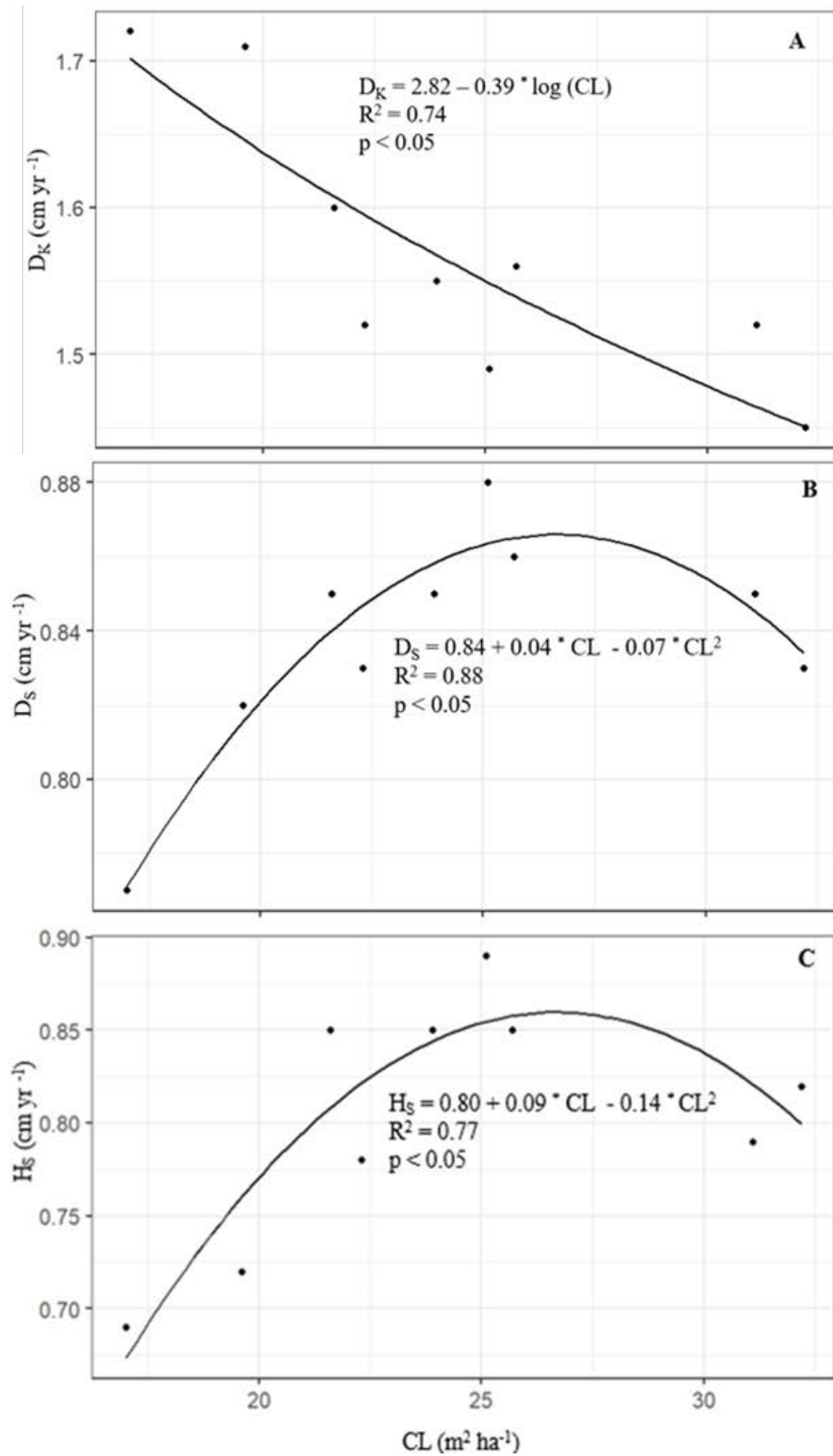
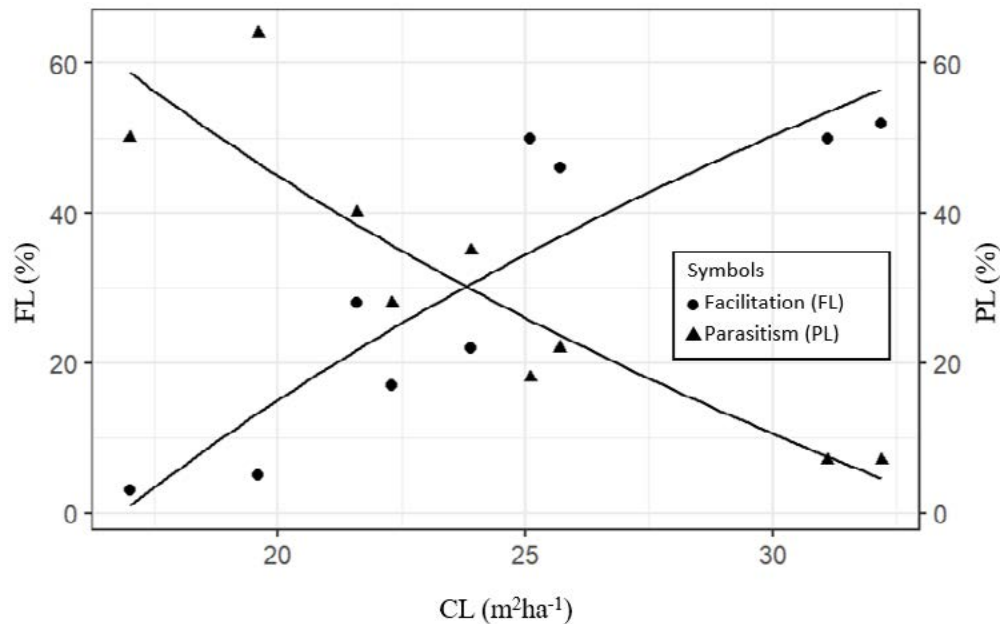


Figure 6. Response of ‘iliahi and koa to competition increased: (A) PAI of koa stem diameter ( $D_K$ ); (B) PAI of ‘iliahi stem diameter ( $D_S$ ); (C) PAI of ‘iliahi height ( $H_S$ ). CL refers to basal area as the indicator of competition.



**Figure 7. Higher competition (a) increased facilitation but (b) mitigated parasitism. CL refers to basal area as the indicator of competition.**

important silvicultural aspects that should be considered to support ‘iliahi reforestation. This project also synthesized the previous two studies about the effectiveness of different reforestation strategies to accelerate ‘iliahi recovery in the natural habitat. Data from the first and second projects were re-organized to develop a structural model using path analysis (Figure 5). This method combined various mathematical expressions, computer algorithms, and statistical approaches to describe the relationship among variables. It could generate a coefficient that denoted the relative contribution of a specific aspect on the behavior of a particular object (Table 1). It also provided a quantitative indicator of the relative influence of various biotic and abiotic factors affecting ‘iliahi growth.

## KEY FINDINGS / ACCOMPLISHMENTS

### Plant Competition

Competition significantly affected the growth

of ‘iliahi and koa but their responses were substantially different. Koa growth responses decreased with increased competition, while ‘iliahi growth increased with competition before declining at basal area > 26 m<sup>2</sup> ha<sup>-1</sup> (Figure 6). ‘iliahi showed an asymmetric response to competition which confirmed a trade-off between light requirements and root parasitic connections. Higher competition also increased facilitation but mitigated parasitism (Figure 7). The importance of facilitation and parasitism are balanced at a moderate level of competition (Figure 7). This corresponded to the highest growth of ‘iliahi but allowed for adequate growth of koa.

### ‘iliahi-Host Ratio

Three categories of relative host density (RHD) were recorded based on the different ‘iliahi-host plant ratio: RHD1 (host < ‘iliahi), RHD2 (host = ‘iliahi), and RHD3 (host > ‘iliahi). Different RHD resulted in significant differences in growth of ‘iliahi and host

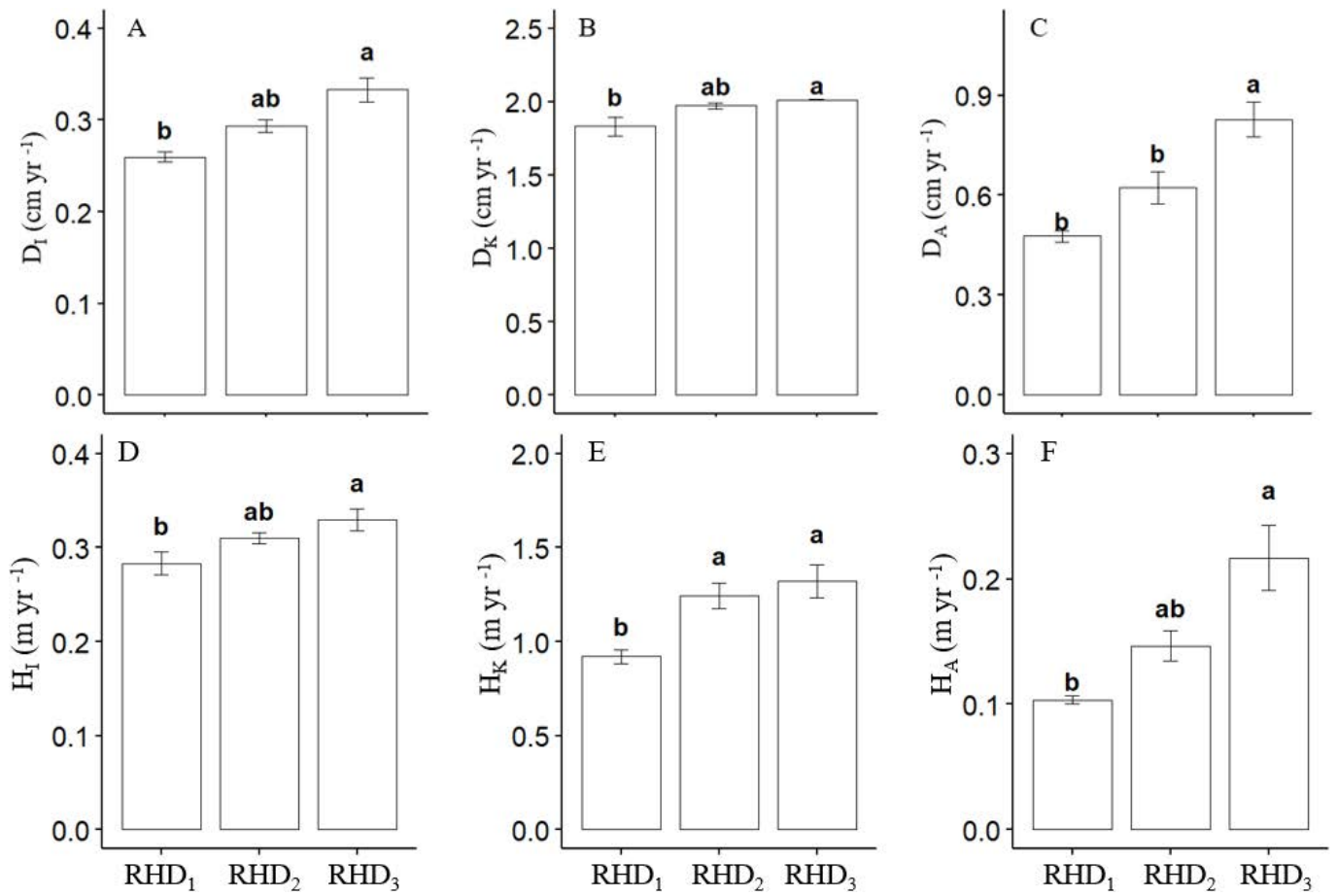


Figure 8. Growth comparison of ‘iliahi and host plants among relative host density (RHD): (A)  $D_{30}$  growth of ‘iliahi; (B) DBH growth of koa; (C)  $D_{30}$  growth of ‘a’ali’i; (D) Height growth of ‘iliahi; (E) Height growth of koa; (F) Height growth of ‘a’ali’i. Different letters above barplot indicated a significant result. RHD<sub>1</sub> (‘iliahi>host), RHD<sub>2</sub> (‘iliahi=host), and RHD<sub>3</sub> (‘iliahi<host).

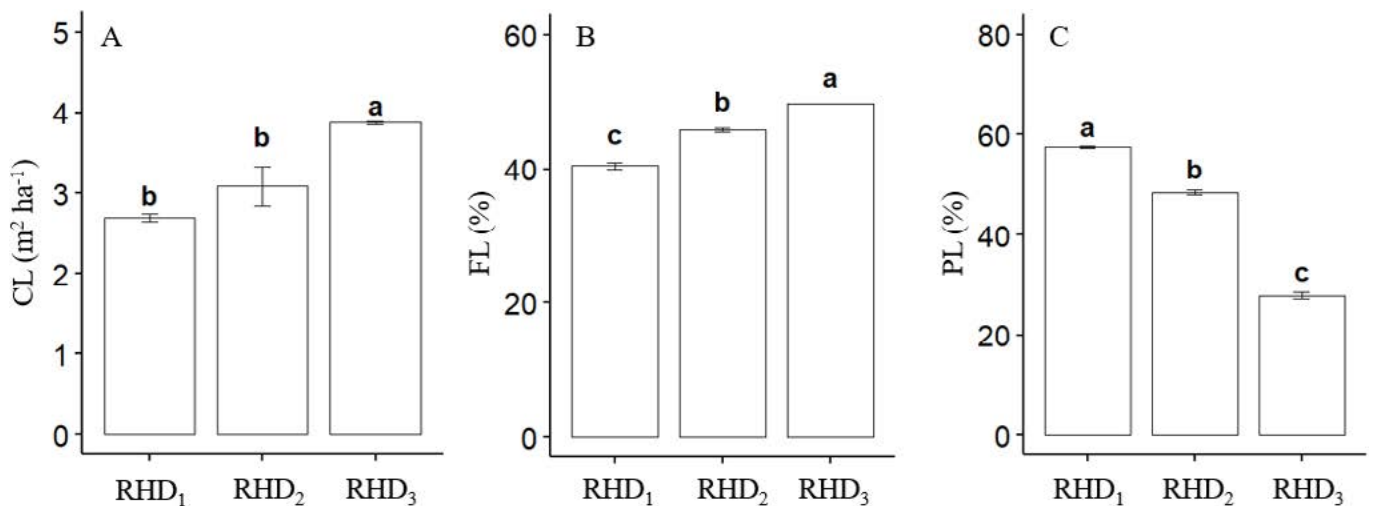


Figure 9. Comparison of ‘iliahi-host interactions among relative host density (RHD): (A) Competition; (B) Facilitation; (C) Parasitism. Different letters above barplot indicated a significant result.

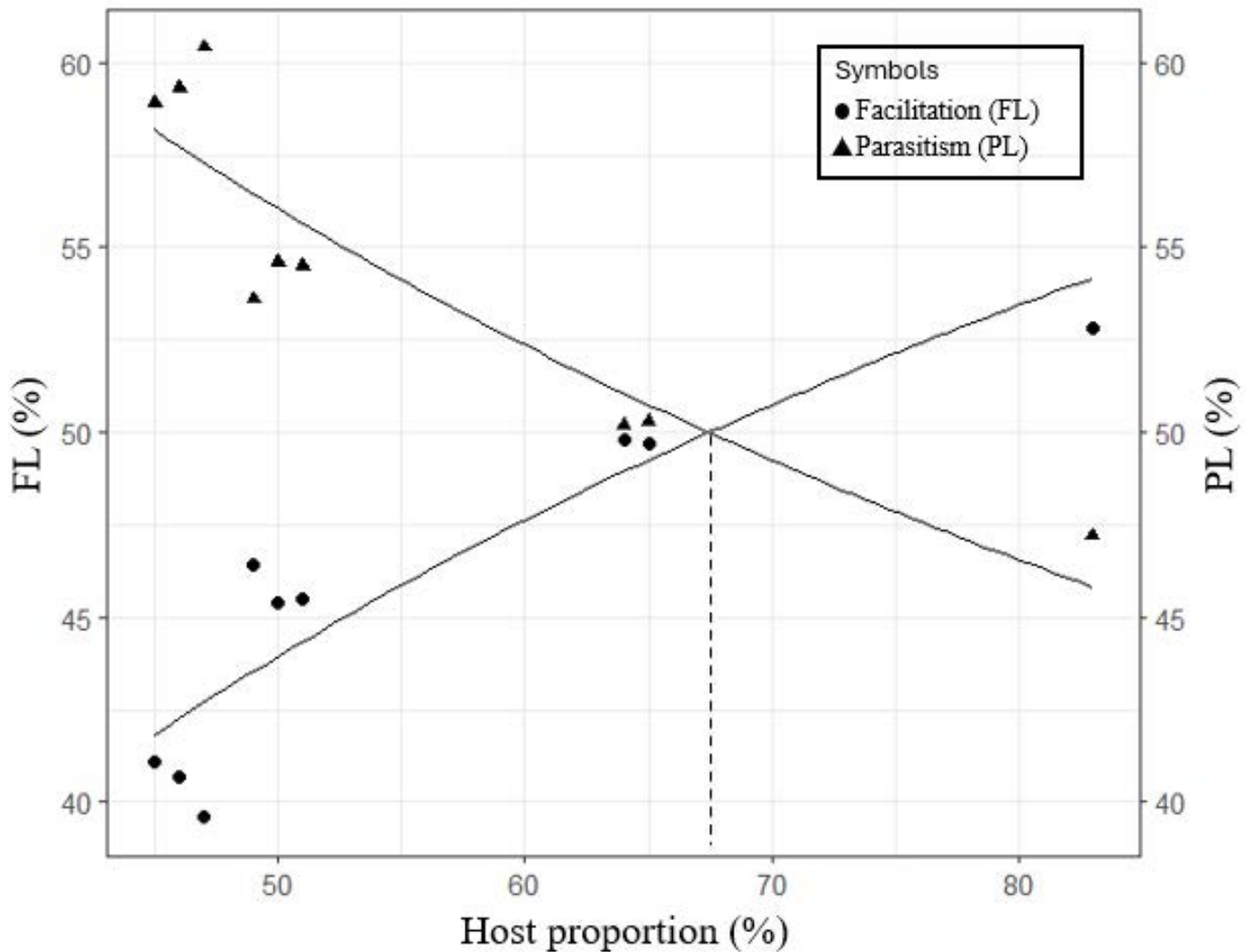


Figure 10. Ideal host-to-'iliahi proportion to balance facilitation and parasitism

(Figure 8). 'Iliahi showed better growth when the host was more abundant. Increasing host-to-'iliahi ratio significantly increased competition and facilitation but decreased parasitism (Figure 9). Foliar %N of 'iliahi also increased with increasing host abundance but foliar %K decreased (Table 2). RHD3 (host>'iliahi) provided best growth for all species with an optimum host-to-'iliahi ratio of 2:1 to balance facilitation and parasitism (Figure 10)

### Factors Limiting 'Iliahi Growth

The developed model indicated a good fit to explain the relationship between biotic interactions and the growth of 'iliahi (Table 3). Planting time (PT) and planting mixture (PM) were the most important factors for growth of 'iliahi (IG) (Figure 11 and Table 4). However, the relative contribution of both factors was substantially different. PT directly affects growth while PM affects growth through an indirect relationship by determining the level

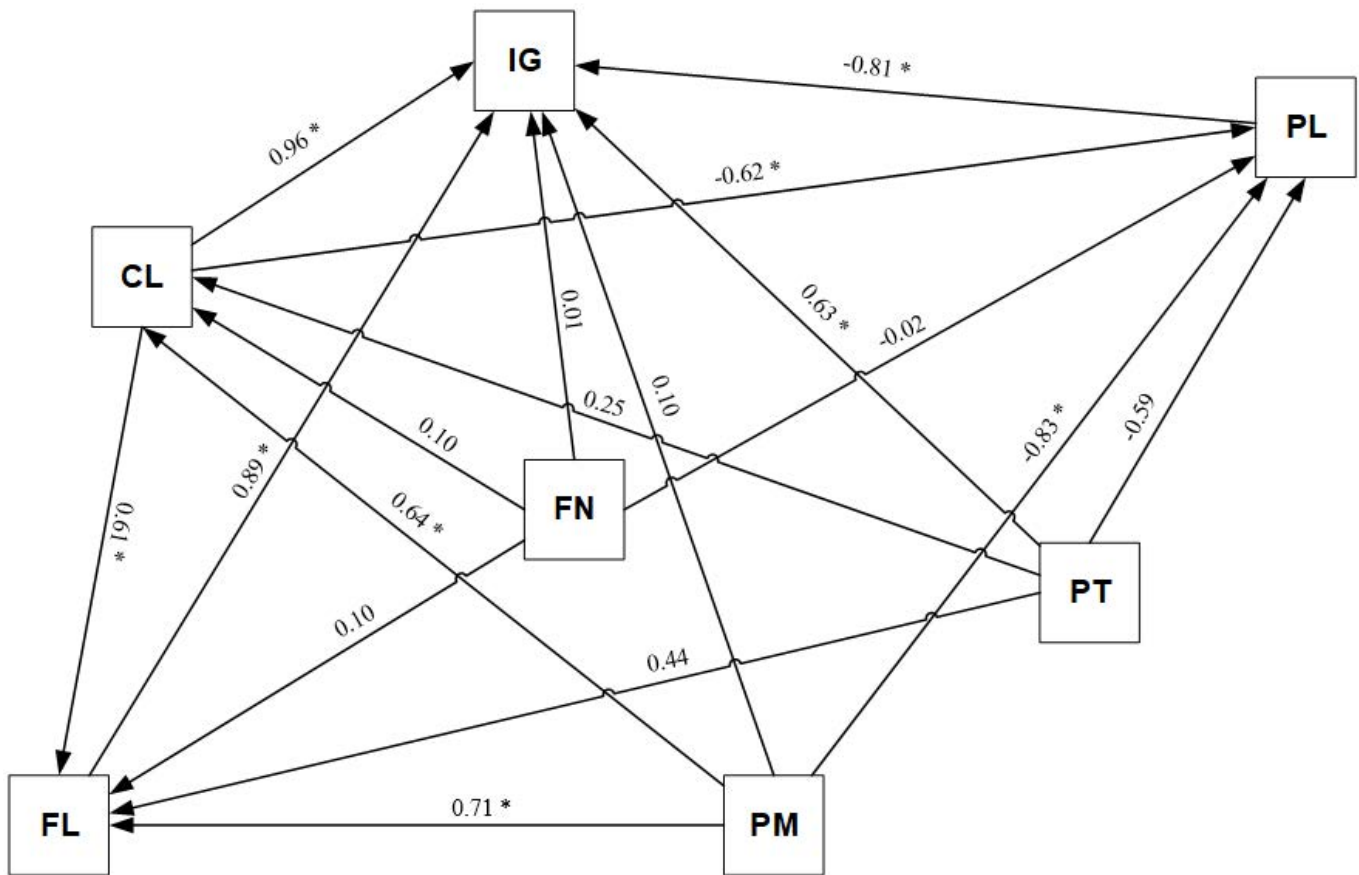


Figure 11. Path diagram demonstrating the relative influences of silviculture strategies on the growth of ‘iliahi in Hawaiian-restored tropical dry forests. Description: PT (planting time), PM (planting mixture), FN (proportion of N<sub>2</sub>-fixing to non-fixing host), CL (competition), FL (facilitation), PL (parasitism), IG (‘iliahi growth). The symbol \* indicated a significant standardized path coefficient

Parameters	Symbol	Unit	Description
Planting Time	PT	-	Binomial variables: 0 = Planting host and ‘iliahi simultaneously 1 = Pre-establishing host before ‘iliahi planting
Planting Mixture	PM	%	The proportion of host-to-‘iliahi in a mixed stand
N <sub>2</sub> fixing Proportion	FN	%	The proportion of N <sub>2</sub> -fixing host compared to non-fixing host
Competition	CL	%	Total basal area at the stand level
Facilitation	FL	%	Facilitation level from host to ‘iliahi in the mixed stand
Parasitism	PL	%	Parasitism level from ‘iliahi to host in the mixed stand
‘Iliahi Growth	IG	cm yr <sup>-1</sup>	Periodic annual increment in diameter of ‘iliahi

Table 1. List of variables to develop a structural equation model.

Parameters	Unit	RHD <sub>1</sub>	RHD <sub>2</sub>	RHD <sub>3</sub>	p-value
N	%	2.21 ± 0.04 a	2.58 ± 0.03 a	3.07 ± 0.17 b	0.002 *
P	%	0.15 ± 0.01 a	0.17 ± 0.01 a	0.14 ± 0.02 a	0.260 <sup>ns</sup>
K	%	2.64 ± 0.01 a	2.44 ± 0.07 b	2.15 ± 0.02 c	0.001 *
Ca	%	0.56 ± 0.01 a	0.55 ± 0.05 a	0.64 ± 0.03 a	0.228 <sup>ns</sup>
Mg	%	0.28 ± 0.02 a	0.28 ± 0.02 a	0.26 ± 0.00 a	0.831 <sup>ns</sup>
S	%	0.16 ± 0.00 a	0.16 ± 0.00 a	0.16 ± 0.00 a	0.271 <sup>ns</sup>

Table 2. Comparison of % foliar nutrient concentration of ‘iliahi across RHD.

Parameters	Symbol	Values	Categories
<b>Validity and Reliability Variables</b>			
Cronbach's Alpha	CA	0.76	Reliable
Average Variance Extraction	AVE	0.89	Valid
<b>Model Fit Indices</b>			
Chi-Square Test (p-value)	$\chi^2$	0.25	Good Fit
Root Mean Square Error of Approximation	RMSEA	0.02	Good Fit
Standardized Root Mean Square Residual	SRMR	0.01	Good Fit
Comparative Fit Index	CFI	0.98	Good Fit
Tucker-Lewis Index	TLI	0.97	Good Fit
Coefficient of Determination	R <sup>2</sup>	0.92	Good Fit

Table 3. Results of goodness of fit test model

Variables	Direct effect	Indirect effect			Total effect
		CL	FL	PL	
PT	0.63*	0.25	0.44	-0.59	0.73*
PM	0.10	0.64*	0.71*	-0.83*	0.62*
FN	0.01	0.10	0.10	-0.02	0.19

Table 4. The relative effect of silviculture strategies on ‘iliahi growth based on the standardized path coefficient. Description: PT (planting time), PM (planting mixture), FN (proportion of N<sub>2</sub>-fixing to non-fixing host), CL (competition), FL (facilitation), PL (parasitism), IG (‘iliahi growth). The symbol \* indicated a significant result at p-value <0.05.

of competition, facilitation, and parasitism. Pre-established hosts before planting 'iliahi offered better growth of 'iliahi since hosts can support early stages of root parasitic connection. However, we must also maintain the host abundance more than 'iliahi to stabilize facilitation and parasitism for better 'iliahi growth.

### **PARTNERS / COLLABORATORS**

Kealakekua Mountain Reserve

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# Host Associations and Environmental Drivers of 'Iliahi Establishment and Drought Performance

## INVESTIGATORS

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## BACKGROUND / JUSTIFICATION

With a lack of abundant seed sources and natural regeneration, 'iliahi is currently facing severe restoration challenges that may be exacerbated in future climates. Importantly, due to 'iliahi's reliance on a host for additional water acquisition through root haustoria, increased incidence and severity of drought may have significant negative impacts on 'iliahi performance and physiology compared to non-hemiparasitic tree species. However, 'iliahi performance is likely largely dependent on the host's ecophysiological responses to climate, as well as the environmental conditions the 'iliahi are established in.

The overall goal of my Ph.D. dissertation proposal is to improve our understanding of 'iliahi and host-'iliahi relationships under different microenvironmental conditions, to help inform management strategies that maximize growth and survival while minimizing the potential for water stress.

## PROJECT 1: EARLY ESTABLISHMENT, GROWTH, AND PHYSIOLOGICAL PERFORMANCE OF UNDERPLANTED 'ILIAHI ACROSS FINE-SCALE MICROSITE VARIATION IN A NATIVE HAWAIIAN FOREST

**Objective: Understanding the influence of microenvironmental variables on underplanted 'iliahi seedling performance and water status.**

- The goal of this objective is to determine a suite of optimal environmental conditions for 'iliahi, that could be emulated in future 'iliahi plantings.

### Approach:

- This project began in the summer of 2023 at Waipunalei (Parker Ranch), in an existing mature mixed forest comprised of primarily koa and 'ōhi'a. We underplanted 120 'iliahi seedlings under a gradient of microenvironmental conditions, helped to create through multiple crop-tree release thinnings for selected canopy koa. Individual tree cages were installed to protect planted

seedlings from feral ungulate browse damage trees.

- Canopy openness and stand basal area were measured at the time of planting.
- In the summer of 2024, we conducted detailed tree-level surveys of microenvironment providing data pertaining to: understory vegetation class cover, microtopography (slope and aspect), soil chemistry, distance to nearest host tree, species of nearest host tree, and DBH of nearest host tree. Repeated instantaneous measurements of soil moisture are collected every 6 months, and will be averaged at tree-level to analyze water relations.
- 'Iliahi growth performance (stem height, root collar diameter) has been measured every 6 months since project initiation. Physiological parameters of plant performance have also been conducted, with chlorophyll fluorescence measured every 6 months and midday water potential measured every 12 months. These tree-level characteristics are being analyzed for potential relationships with the microenvironmental conditions of each tree planting microsite.

### **Preliminary results:**

- In the time since outplanting, only 2 of 120 seedlings have succumbed to mortality. Height growth for the first 2 years has been rapid, averaging 1.48 m (Figure 1). Average 2-year diameter growth of seedlings was 1.4 cm.
- As of August 2025, we have detected that only initial seedling size at the time of outplanting (height and root collar

diameter) as well as soil moisture have had significant influences on 'iliahi growth.

- Canopy openness, microtopography, understory vegetation competition indices, distance to nearest hosts, and nearest host DBH/species have not yet shown significant impacts on 'iliahi growth.
- We have detected a negative effect of increased soil moisture on both seedling height and diameter, with an interaction effect between soil moisture and time (Figure 2). As the seedling grows older, the negative relationship between growth and soil moisture grows stronger.
- As this site receives 2,000-2,500 mm of precipitation a year on average, this may indicate that excessive soil moisture in a non-moisture-limited system may have negative impacts on height growth. However, this may also be a result of drier soil driving increasingly negative water potentials for shallow-rooted 'iliahi seedlings (Figure 3).
- The hemiparasitic water movement strategy requires supporting a water potential gradient where the hemiparasite has a more negative water potential than the host, preserving a gradient and allowing the water to move from less to more negative.
- Mature, deep-rooted hosts are more buffered from shallow soil drying than the shallow-rooted 'iliahi, so microsite dryness may strengthen this gradient and allow for greater hemiparasitic



Figure 1: Representative rapid growth of 'iliahi in the Waipunalei Forest Reserve from year 1 post-outplanting to year 2. 'Iliahi seedlings have been grown in individual protective tree cages to prevent feral ungulate damage.

### 'Iliahi height vs. soil moisture

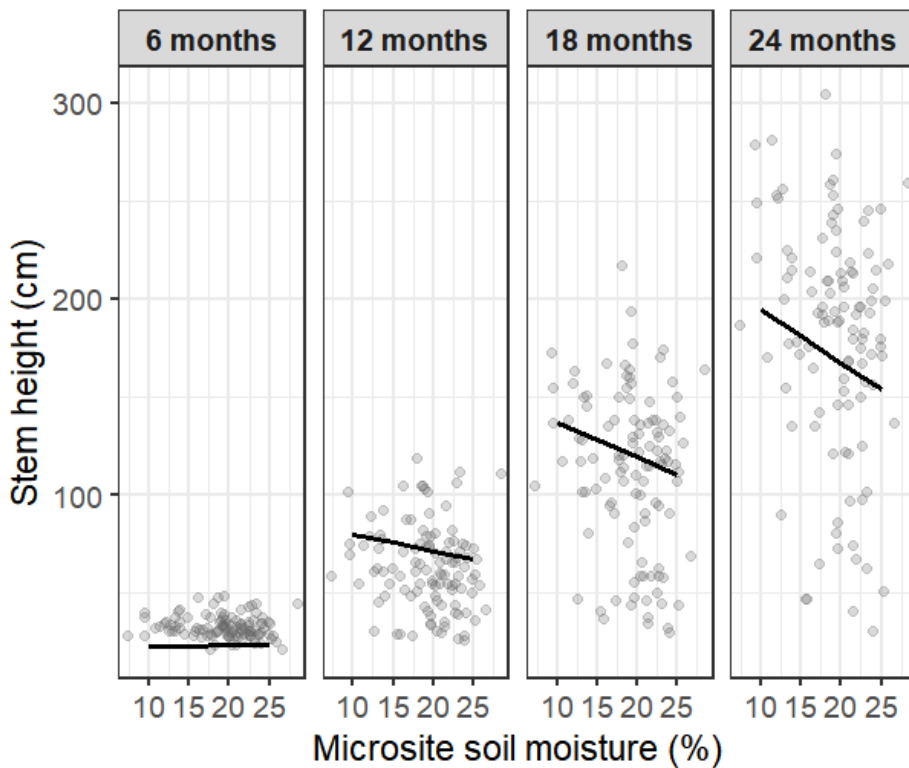


Figure 2: a) Raw soil moisture data vs. 'iliahi stem height (grey circles) with the linear mixed-effects model overlayed (black line) showing the negative correlation of growth with soil moisture that becomes stronger over time.

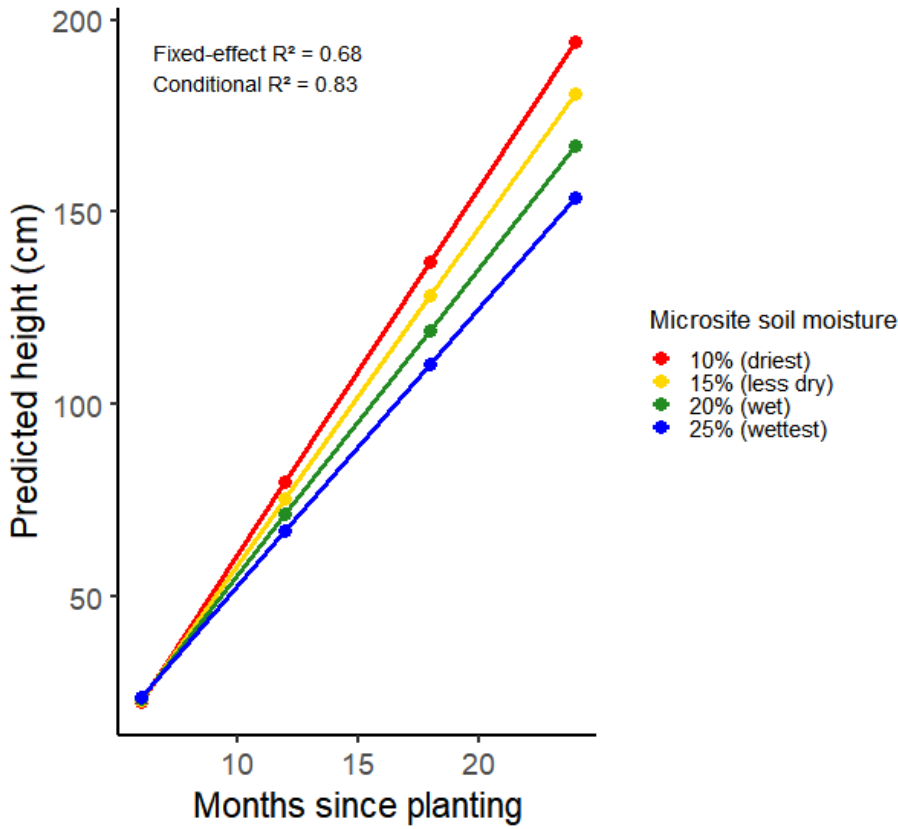


Figure 2: b) Predicted results of the linear-mixed effects model (height ~ soil moisture \* time + initial height + initial diameter) for representative soil moisture categories ranging from driest (red) to wettest (blue).

### Midday Water Potential vs Soil Moisture Mixed Model Fit (Height held at mean)

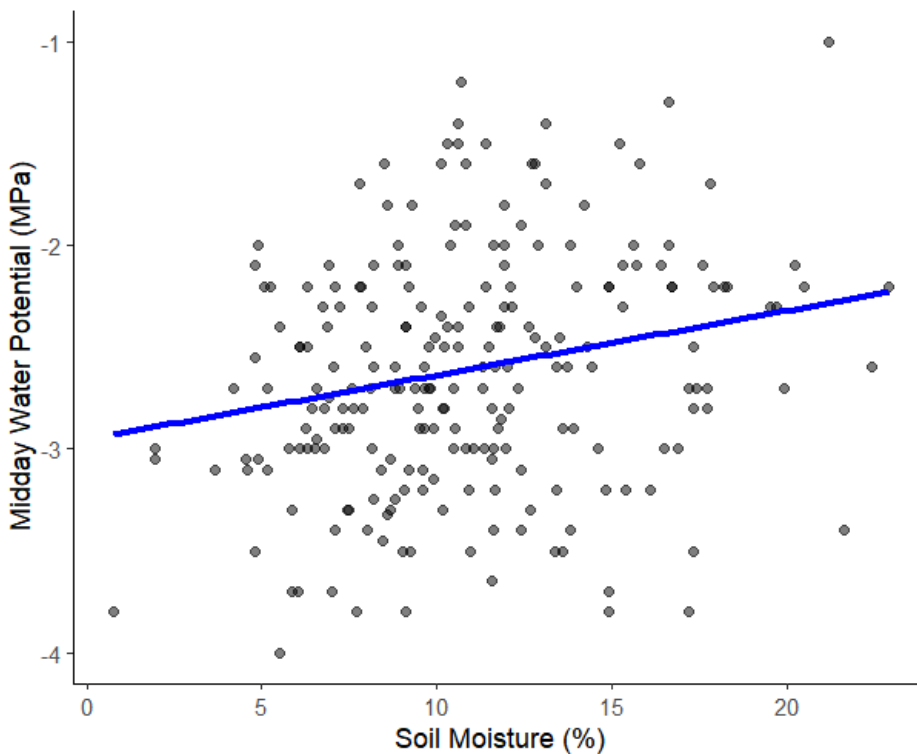
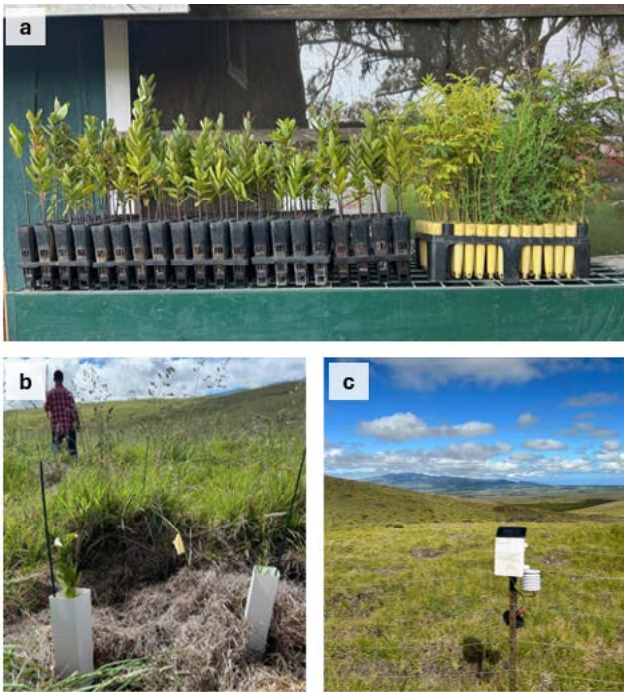


Figure 3: Drier microsites have more negative midday water potentials, with raw data points represented by grey circles and the significant linear model overlaid (blue line).



**Figure 4:** a) 'Iliahi and mixed host species (koa, koai'a, Monterey cypress) prior to outplanting. b) Milk carton wind and desiccation protection established for each planted seedling. c) Climate dataloggers installed at each plot.

transfer, potentially contributing to greater 'iliahi growth in drier planting sites.

- Average midday water potential for all 'iliahi seedlings was -2.28 and -2.9 MPa in August 2024 and 2025, respectively, which is noticeably lower than other tropical dry forest tree species including many Fabaceae species that may maintain midday water potential levels closer to -1.0 MPa when not stressed.
  - Field measurements of surrounding koa at Waipuanlei ranged from -0.5 to -1.1 MPa.

## **PROJECT 2: OPTIMIZING HOST PAIRINGS ACROSS MICROCLIMATIC GRADIENTS FOR 'ILIAHI RESTORATION**

### **Objective: Improve our understanding of the interactive effects of planting site microclimate and host species on initial 'iliahi performance.**

- The goal of this objective is to determine which host is best suited for co-planting with 'iliahi across the climate gradients, to inform future restoration planting strategies for non-forested areas.

### **Approach:**

- Six plots spanning from 1490 – 1950 m in elevation were planted in May 2024 in Parker Ranch pastureland on Mauna Kea to initiate this study, using already established fenced exclosures.
- A total of 40 'iliahi were planted per plot, either alone (control) or with a host 0.5 m away (host species: koa, koai'a, and Monterey cypress, Figure 4a). A boundary of a'ali'i were also planted to reduce edge effects. Each plot was a complete randomized design, with a minimum of 3m spacing between replicates.
  - Milk cartons were installed with bamboo stakes for each planted seedling for wind and desiccation protection (Figure 4b).
  - Initial height and diameter were recorded at the time of outplanting.
- Climate dataloggers are installed at each plot, continuously monitoring air temperature, relative humidity, soil temperature, and soil moisture content at 30-minute intervals (Figure 4c).
- In May 2025 we collect seedling performance data including 1-year height and diameter, chlorophyll fluorescence, and midday water potential

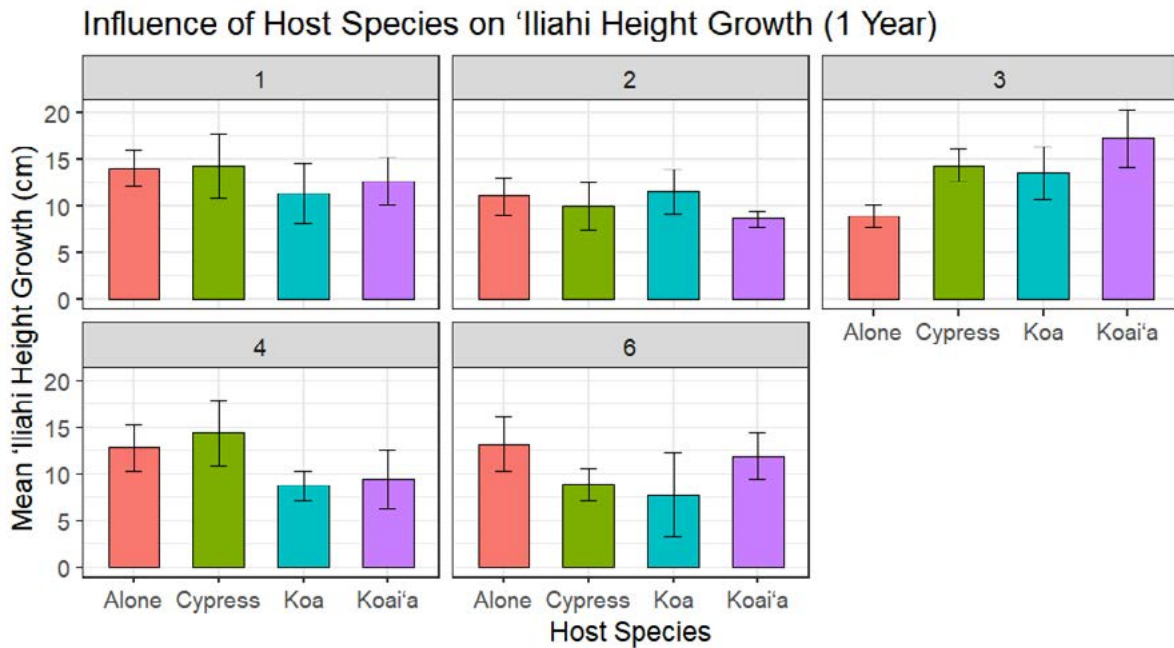


Figure 5. Average 'Iliahi height growth (+/- S.E.) grouped by co-planted host species across the five pastureland plots (#5 was omitted due to extensive mortality). Red: alone ('Iliahi planted by itself), green: co-planted with cypress, teal: co-planted with koa, purple: co-planted with koai'a. While trends differed across the plots, no significant impact of host species or host\*microclimate has emerged in models at the 1-year mark.

measurements for both 'Iliahi and co-planted hosts. This will be repeated in May 2026 prior to final analysis.

**Preliminary Results:**

- Across all six plots, there is a wide range in microclimatic conditions (soil water content, soil temperature, air temperature, and air humidity) with no apparent correlation between elevation and soil water content emerging currently.
- One-year post outplanting, there was a range in 'Iliahi performance and conditions across all sites, however in five of six sites there was still limited mortality with only 8/200 trees succumbing to

mortality. One plot experienced significant mortality (100% host death, 67.5% 'Iliahi death) likely due to wind exposure

causing extensive blowdown, so this plot was removed from the study analysis.

- As of now, there is no clear significant effect of host species or interaction between host and microclimate on 'Iliahi growth (Figure 5) or physiological performance. This was expected as it takes time for 'Iliahi to become established and form haustorial connections with co-planted hosts, and there will more likely be an effect that emerges in 2-year data.

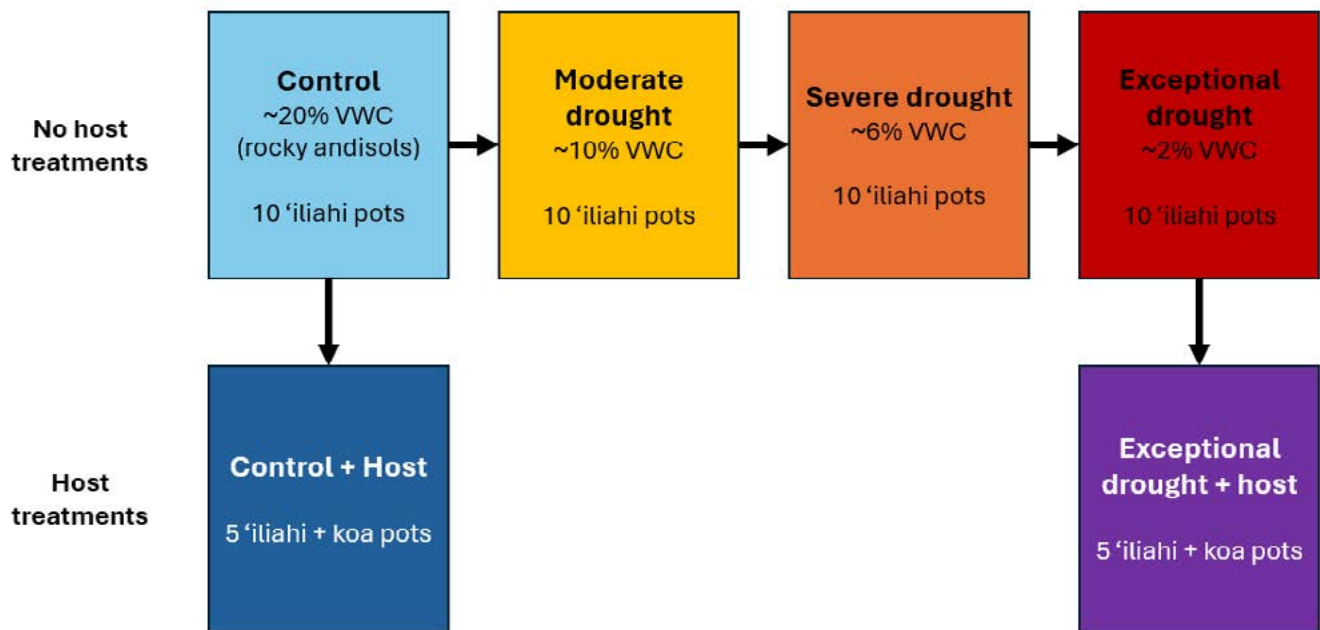


Figure 6. Final controlled drought experimental design showing both 'iliahi alone (no host treatments) and the supplemental host treatments included for control and exceptional drought levels.

### PROJECT 3: INTERACTIVE EFFECTS OF DROUGHT INTENSITY AND HOST ASSOCIATION ON THE PHYSIOLOGICAL PERFORMANCE OF 'ILIAHI

**Objectives:** 1) Test how 'iliahi drought responses change across increasing drought severity levels. 2) Assess if host presence alters 'iliahi performance under exceptional drought.

- The goal of this objective is to determine how water deficit and the presence of a host impacts 'iliahi water and carbon dynamics, to inform future restoration planting locations and timing to give projects the best chance at 'iliahi survival and success.

#### Approach

- In 2024, we began growing multiple cohorts of 'iliahi seedlings at the Purdue University greenhouses. After six months of growth, we sowed imbibed koa seeds directly into a subset of 'iliahi pots to

facilitate the formation of haustoria.

Koa and 'iliahi were grown together for approximately 8 months. In Fall of 2025, koa-'iliahi pairs as well as individual non-hosted 'iliahi from the same cohort were transplanted to a larger ~3-gallon pots filled with 3:1 perlite to BM6 potting mix and allowed one month to acclimate to the new pot. Koa-'iliahi pairs were visually inspected for haustorial connections at the time of transplanting and pairs with no evidence of haustoria were omitted.

- Due to a mass koa mortality event that occurred in Summer 2025, only 10 koa-'iliahi pairs were able to be included in the final study design.
- A pilot drought experiment was conducted in April/May of 2025 and data was used to inform the dry-down and data collection process of the final experiment. The final drought experiment was conducted in the Fall of 2025.

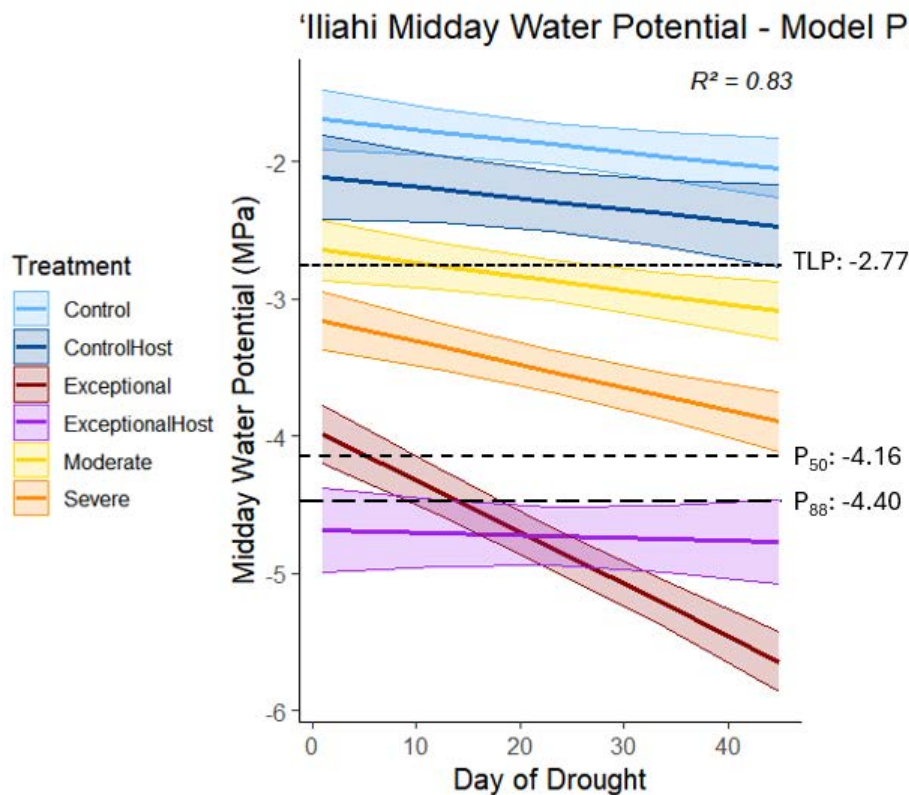


Figure 7. Model predictions of ‘iliahi midday water potential vs. day of drought. Dark blue and dark red lines indicate the drought+host treatments where koa was paired with ‘iliahi. Dashed lines indicate lab-quantified estimates of hydraulic thresholds for ‘iliahi including turgor loss point (TLP), P50 (50% loss of hydraulic conductivity) and P88 (88% loss of hydraulic conductivity).

- This study included four drought levels: control: well-watered, (20% volumetric water content (VWC)), moderate drought (10% VWC), severe drought (6% VWC) and exceptional drought (2% VWC). For each of these drought levels, 10 pots containing individual ‘iliahi were included. For the control and exceptional drought treatments, 5 koa-‘iliahi paired pots were included per level (Figure 6).
- Pots were weighed and watered every day up to their respective drought thresholds, and maintained at these thresholds for 45 days. Over that time, repeated measurements of physiological performance including midday water

potential, stomatal conductance, transpiration, dark- and light-adapted fluorescence, and leaf relative water content were conducted on both ‘iliahi and paired hosts (when applicable).

- At the drought end, ‘iliahi and koa were destructively harvested and will be analyzed in Spring 2026 for nonstructural carbohydrates and osmotic adjustment.

**Preliminary Results:**

- Midday water potential declined stepwise with increasing drought severity, indicating progressive hydraulic stress with water limitation (Figure 7). Exceptional drought without a host

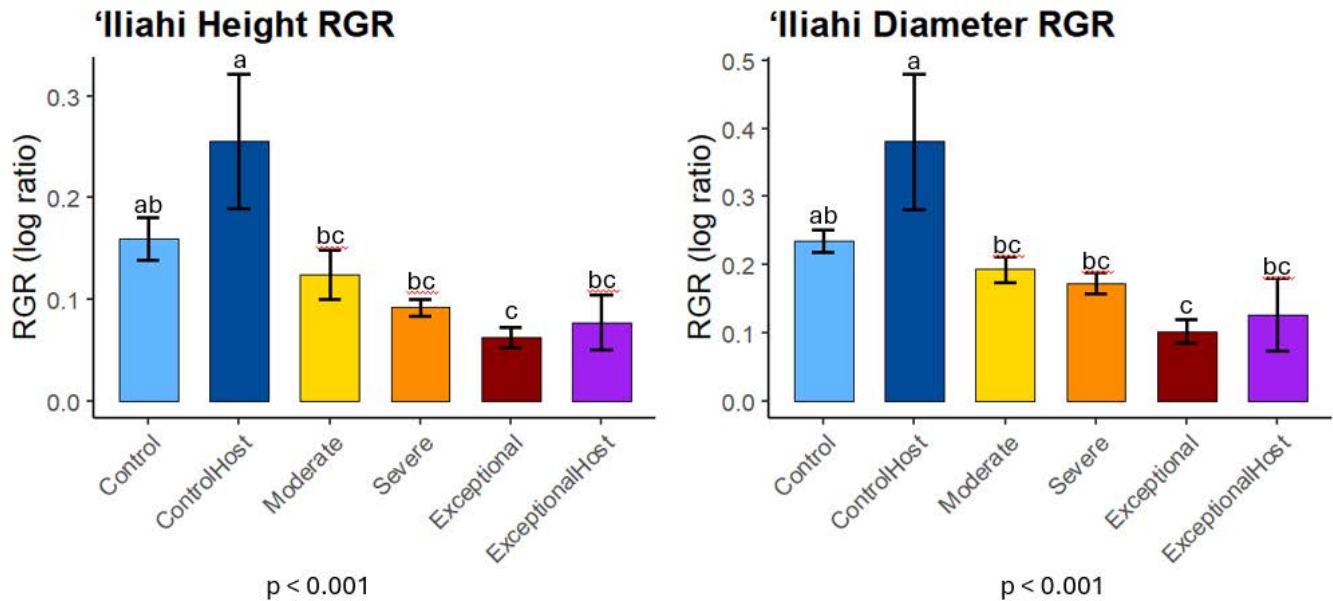


Figure 8. Relative growth rates for height (left) and diameter (right) of 'Iliahi under drought and host treatment levels (+/- S.E.). Different letters indicate significant differences among treatments based on post hoc pairwise comparisons ( $p < 0.05$ ).

showed the steepest decline (-4 to -5.7 MPa), suggesting loss of hydraulic function over time.

- Interestingly, the exceptional drought treatment + host started with the lowest water potential, but remained stable throughout the 45 days of drought, suggesting the host may buffer further hydraulic decline even when operating below physiological thresholds of hydraulic stress (i.e. TLP, P50).
- We found that 'Iliahi closes stomata more (decreasing stomatal conductance) as drought severity intensifies, indicating a drought avoidance physiological strategy. The well-watered control 'Iliahi with the koa host showed increased conductance over time, reflecting enhanced water

access compared to the control 'Iliahi grown alone.

- Even under exceptional drought conditions, the exceptional + host 'Iliahi also increased conductance, likely due to hydraulic support from the koa.
- Dark-adapted fluorescence different among treatments, but not in a biologically significant way, with fluorescence remaining in healthy ranges even under exceptional drought.
  - This is interesting as it suggesting hydraulic stress far precedes photochemical collapse in 'Iliahi.
- By the end of the drought, the well-watered control + host 'Iliahi grew the most in both height and diameter (Figure

8), significantly more than all drought treatments. Under exceptional drought with no host, growth was the lowest. The presence of a host under exceptional drought provided some growth improvement, but not a full recovery.

- Interestingly, moderate and severe drought height and diameter growth were not significantly different than the control (no host) 'iliahi growth, indicating substantial drought tolerance through physiological mechanisms. These drought treatments likely relied heavily on using NSC reserves and osmotic adjustment to maintain growth and survive even with limited stomatal conductance.

### **PARTNERS / COLLABORATORS**

- Parker Ranch – Zachary Judd (Forestry Manager) and Bear Ingram
- USDA Forest Service Institute of Pacific Islands Forestry
- USDA Forest Service – Emily Thyroff
- Hāloa 'Āina – Tawn Speetjens
- Waimea State Tree Nursery – Cheyanne Rapoza
- University of Hawai'i at Mānoa – JB Friday, Travis Idol

### **PUBLICATIONS**

French, K.L. "Roots of Resilience: Restoring 'Iliahi and the Connections That Sustain Us." Forestry Source, Society of American Foresters. February 2026.

# Genetic Diversity of Sandalwoods in Hawaiian Forests

## INVESTIGATORS

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**Clifford Morden**, Professor, School of Life Sciences, University of Hawai'i at Mānoa

## BACKGROUND / JUSTIFICATION

Sandalwood is a hemiparasitic tree of the genus *Santalum* L. (Santalaceae). Two lineages of Hawaiian sandalwoods (‘iliahi) are believed to have originated from the South Pacific. A red flowered clade composed of *Santalum pyrularium* A. Gray, *Santalum freycinetianum* Gaudich., and *Santalum haleakalae* Hillebr. This clade is most closely related to species of *Santalum* L. from French Polynesia and Ogasawara Islands. The white flowered clade composed of *Santalum paniculatum* Hook. & Arn. and *Santalum ellipticum* Gaudich. are most closely related to species of Australasian origin. *Santalum involutum* H. St. John is a federally listed endangered species endemic to Kaua'i likely of an ancient hybrid origin. The introduced Indian sandalwood (*Santalum album* L.) readily hybridizes with the endemic *S. ellipticum* Gaudich. These hybrids spread readily and pose a threat to endemic members of the genus where their ranges intersect.

As part of his PhD research in the School of Life Sciences at the University of Hawai'i at Mānoa, Solomon Champion studied the genetics of the endemic Hawaiian sandalwoods.

## PROJECT OBJECTIVES

- Ascertain the degree of interspecific hybridization and introgression among Hawaiian sandalwood species and the introduced Indian sandalwood (*Santalum album* L.).
- Develop molecular marker assisted protocols for evaluating introgression and hybridization within Hawaiian sandalwood species for the purpose of verifying stock types prior to seed orchard or plantation implementation.
- Investigating the evolutionary relationships between biogeography, population genetics, speciation and ecosystems services (i.e., pollination and dispersal).
- Explore the links, if any, between genotype and santalol production within sandalwood heartwood.

## APPROACH

### Genotyping by Random Amplicon Sequencing, Direct (GRAS-Di)

Randomized genotyping allows for greater representation of the entire genome, compared to microsatellites or DNA barcoding. The breadth of the sequencing done when genotyped allows for individual mother trees to be compared directly across multiple single nucleotide polymorphisms (SNP). These differences between SNP in individuals, populations, and species are used to measure genetic distance (FST). All Hawaiian sandalwood species, except for

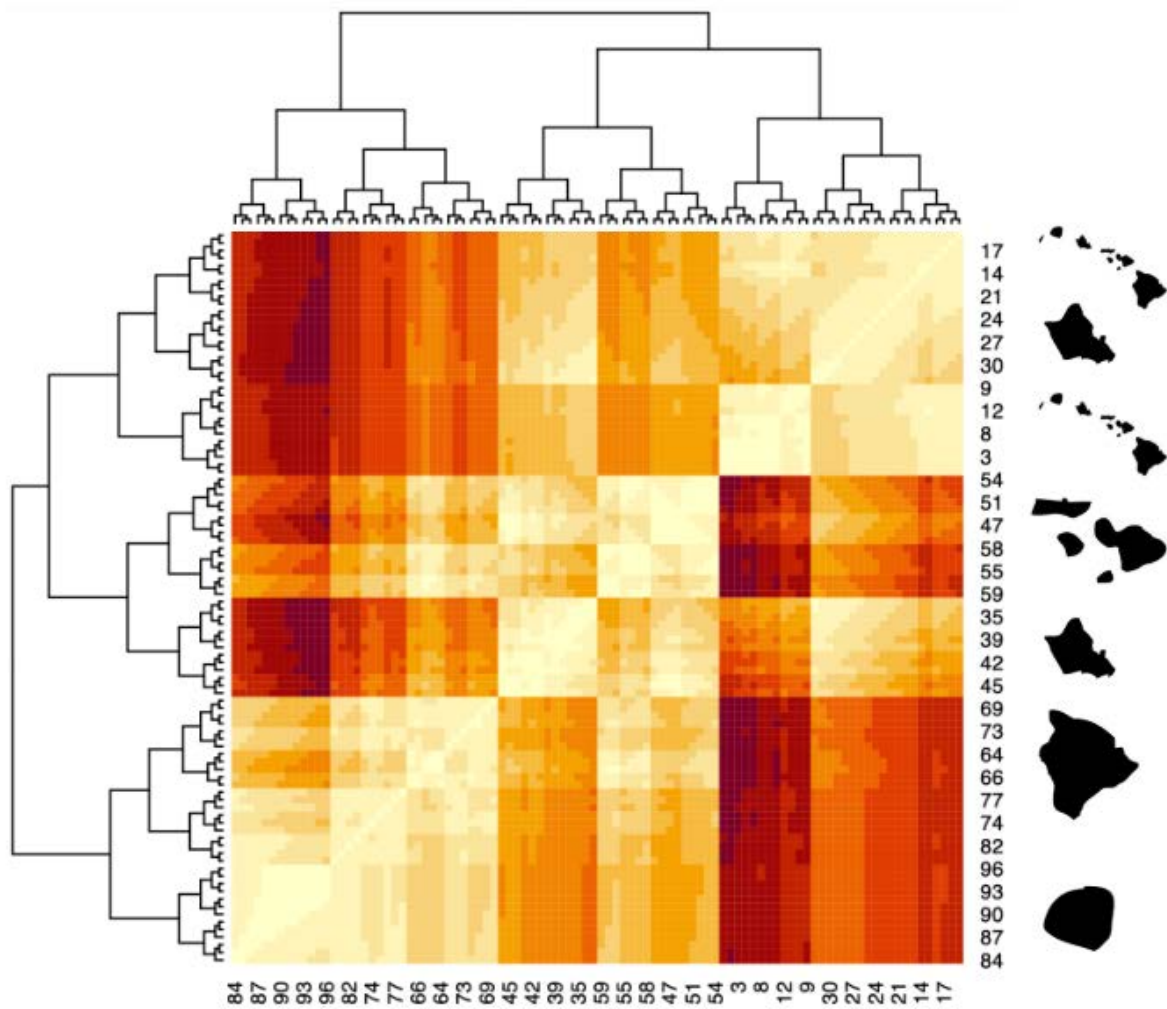


Figure 1. Heat Map of  $F_{ST}$  values across 2,232 GRAS-Di SNPs of 96 individuals. Blocking is annotated to the right of the heat map. With each of the endemic species from Kaua’i, O’ahu, and Maui representing their endemic genotype. *Santalum paniculatum* from Hawai’i Island and *S. ellipticum* from across the main Hawaiian Islands share a consistent genotype. Each island block represents its respective endemic genotype. *Santalum ellipticum* is represented by the blocks adjacent to the entire archipelago.

*S. involutum*, are included in this study; as well as the Indian sandalwood (*S. album*).

**KEY ACCOMPLISHMENTS**

These genomic studies confirm the interspecific gene flow between the two sections of Hawaiian *Santalum*, *Hawaiiensia* and *Solenanthes*; as well as the introduced *S. album*. This study also found the genotypes of *S. ellipticum* and *S. paniculatum* to be shared across Kaua’i, O’ahu, Maui, and Hawai’i Island, suggesting relatively recent

gene flow between the islands, with the Maui samples significantly admixed with the *S. haleakalae* genotype. Repeated hybrid speciation appears to underlie Hawaiian sandalwood biodiversity. All populations of ‘iliahi across the state are admixed with neighboring island species to some degree. It is likely that the coastal Hawaiian sandalwood (*Santalum ellipticum*), ‘iliahialoe, has facilitated significant gene flow throughout the islands in the past. Genotypes

of the presumably derived *S. paniculatum* remain consistent with the coastal 'iliahialoe, suggesting little genetic change during the past 0.5 million years. Older islands have genotypes endemic to those islands, representative of their taxonomy within section Solenantha. On Hawai'i Island it appears that there has not been enough evolutionary time for another genotype to have evolved within section Hawaiiensia for *S. paniculatum* to be considered genetically distinct from *S. ellipticum*. From a tree breeding perspective this effectively increases the capacity of genetic source material available for tree improvement by perhaps 5-6 times; considering each population of *S. ellipticum* from the other islands as potential crosses with Hawai'i Island Santalum.

Historically, the sandalwood trade in the Hawaiian Islands began around 1810. Large areas of forest were effectively overharvested leaving fragmented populations across the archipelago. In its wake people were encouraged to plant Indian sandalwood. Since that time, hybridization between Hawaiian and Indian sandalwood species has been slowly on the rise. Hybrid individuals can be easily observed naturally recruiting all along Lē'ahi crater on O'ahu. Surveys have also revealed individuals of *Santalum album* recruiting in forest reserves in the Southern Ko'olau Range. Potentially jeopardizing seed collecting efforts for *Santalum freycinetianum* reforestation on

O'ahu as well. This genotyping effort gives a clearer view of the genetic diversity of Santalum in the Hawaiian Islands. A broader understanding of the relatedness of the six species presently described from Hawai'i is useful for conservation, reforestation and commercial improvement of stock material.

### **FUTURE PLANS**

Establishing verified Hawaiian sandalwood seed orchards remains a priority. Tree breeding programs for Hawaiian sandalwoods should utilize molecular techniques for vetting seed stock collected from forest reserves as a clear risk of *Santalum album* introgression has been identified.

### **PARTNERS / STAKEHOLDERS / COLLABORATORS**

- GikenBio
- Hāloa 'Āina
- Tropical Hardwood Tree Improvement and Regeneration Center
- Kealakekua Mountain Reserve
- Hawai'i Division of Forestry and Wildlife

# ‘Ōhi‘a Disease Resistance Program

## PRINCIPLE INVESTIGATOR

**Marc Hughes**, Research Plant Pathologist, Institute of Pacific Islands Forestry, USDA Forest Service

## CO-LEADS

**Christian Giardina**, Research Ecologist, Institute of Pacific Islands Forestry, USDA Forest Service

**Ryan Belcher**, Operations Specialist and Coordinator, Akaka Foundation for Tropical Forests

**Nainoa Goo**, Greenhouse and Field Specialist Coordinator, Akaka Foundation for Tropical Forests

## RESEARCH TEAM

**Kenjo Pollman**, Research Associate, Akaka Foundation for Tropical Forests

**Tuimalata Puletiutaoa**, Research Associate, Pacific Cooperative Studies Unit

## ADMINISTRATION & FUNDRAISING

**Rebekah Ohara, PhD**, President and CEO, Akaka Foundation for Tropical Forests

## BACKGROUND / JUSTIFICATION

‘Ōhi‘a (*Metrosideros polymorpha*) is the foundation of Hawai‘i’s native forests and the watersheds that sustain the state’s freshwater resources. Covering nearly one million acres statewide, ‘Ōhi‘a supports diverse native ecosystems and is deeply embedded in Native Hawaiian cultural practices and lifeways.

Rapid ‘Ōhi‘a Death (ROD), caused by the non-native fungal pathogens *Ceratocystis*

*lukuohia* and *Ceratocystis huliohia*, has killed more than one million ‘Ōhi‘a trees across approximately 270,000 acres of Hawai‘i’s forests, posing one of the most significant threats to the state’s native ecosystems.

The ‘Ōhi‘a Disease Resistance Program (‘ŌDRP) was established in 2018 to identify, propagate, and develop ROD-resistant ‘Ōhi‘a to support forest restoration across Hawai‘i.

## PROJECT OBJECTIVES

- Perpetuate and restore ‘Ōhi‘a across Hawai‘i to protect the ecological, cultural, and community systems that depend on this keystone species.
- Identify disease-resistant parent trees and expand genetic representation by collecting, propagating, and screening seedlings, rooted cuttings, and seed from diverse zones and forest conditions across the islands to develop regionally adapted breeding populations.
- Establish common gardens and seed orchards to support germplasm preservation, long-term resistance testing, and climate resilience research.

## APPROACH

The ‘ŌDRP conducts research and collections in forests heavily impacted by ROD across Hawai‘i. Field teams identify surviving ‘Ōhi‘a in affected areas and collect seed and cuttings for propagation and resistance screening. Collections are made from diverse locations statewide, with sites selected based on disease impact, geographic location, and elevation. In collaboration with partner

agencies and seed banks, the program supplements these collections to broaden the range of seed sources and genetic diversity screened for ROD resistance.

To date, over one thousand mother trees have been tested for resistance to ROD, and a subset has demonstrated moderate to high resistance to *Ceratocystis lukuohia*. These individuals will be established in field plantings for long-term evaluation and seed production.

## KEY ACCOMPLISHMENTS

### Completed ROD Screening Trials

- Trial 6: Dec 17, 2024 – Jun 17, 2025  
Screened 297 plants representing 36 genotypes from Hawai'i Island and Maui.
- Trial 7: Feb 26, 2025 – Aug 26, 2025  
Screened 305 plants representing 36 genotypes from Hawai'i Island and Maui.
- Trial 9: Oct 2, 2025 – Jan 2, 2026  
Screened 39 plants representing 9 genotypes from Hawai'i Island. Retesting survivor plants from Trials 6 and 7.

### Ongoing ROD Screening Trials

- Trial 8: Nov 20, 2025 – May 20, 2026  
Screened 226 plants representing 21 genotypes from Hawai'i Island and Maui.

### ROD Experimental Studies

Inoculation Timeline Experiment – Evaluated the incubation period of *C. lukuohia* and pathogen viability on inoculation materials across two replicates.

- Rep 1: 44 plants » Jan–Mar 2025 » Completed.
- Rep 2: 44 plants » Oct 2025–Apr 2026 » Ongoing.



Screening trial at the USDA Forest Service in Hilo.

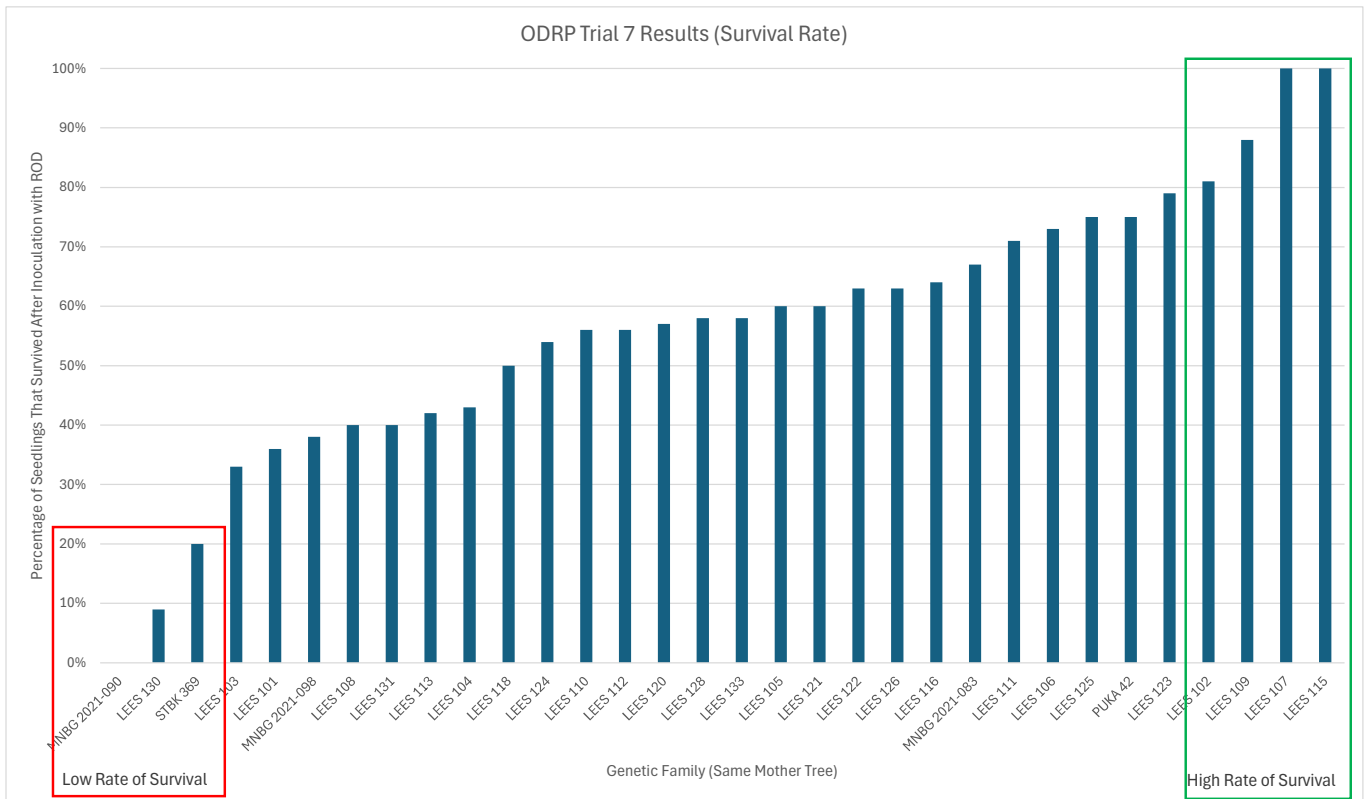
### Seedling Testing

Inside–Outside Pilot Experiment – Compared ROD inoculation outcomes in greenhouse versus outdoor environments across 140 plants in two replicates.

- Rep 1: 60 plants » Mar 19–Sep 17, 2025 » Completed.
- Rep 2: 80 plants » Oct 2, 2025–Apr 2, 2026 » Ongoing.

Root Inoculation Trial – Assessed whether 'ōhi'a can become infected through root wounding across two replicates.

- Rep 1: 100 plants » Aug–Dec 2024 » Completed.
- Rep 2: 100 plants » Jan–Jun 2025 » Completed.



Results showing wilt severity responses of different ‘ōhi’a families following *Ceratocystis* inoculation.

Fungicide Experiment — Evaluating whether two systemic rust fungicides reduce disease progression or increase survival during resistance screening.

- Rep 1: 40 plants » Dec 2025–May 2026 » Ongoing.

**FUTURE PLANS**

**Seedling Resistance Testing**

In collaboration with Hawai’i seed banks and partner organizations, the ‘ODRP will expand seed evaluation from diverse geographic regions across the islands, with the long-term goal of identifying mother trees that consistently produce ROD-resistant offspring to serve as foundational breeding stock for future seed orchards and restoration plantings.

**Keaukaha Military Reserve ‘Ōhi’a Common Garden**

Sixty ‘ōhi’a that passed greenhouse resistance screening were planted in a common garden field trial at Keaukaha Military Reserve, an area with high ROD prevalence. These trees will be monitored over time to evaluate the durability of resistance under natural field conditions. The planting also serves as a prototype seed orchard that may contribute resistant planting stock for future restoration and conservation efforts.

## PARTNERS / COLLABORATORS

- USDA Forest Service – Washington Office  
*(Major financial supporter)*
- State of Hawai'i Division of Forestry and Wildlife  
*(Major financial supporter)*
- University of Hawai'i at Mānoa – College of Tropical Agriculture and Human Resources, Cooperative Extension  
*(Financial supporter)*
- Hawai'i National Guard Environmental Office  
*(Financial supporter)*
- USDA Agricultural Research Service – Pacific Basin Agricultural Research Center
- Laukahi: The Hawai'i Plant Conservation Network
- Purdue University
- Tropical Hardwood Tree Improvement and Regeneration Center
- University of Hawai'i at Hilo – Spatial Data Analysis and Visualization Lab
- Arizona State University – Center for Global Discovery and Conservation Science
- Hawai'i Agriculture Research Center
- Kalehua Seed Conservation Consulting



**Common garden field trial at Keaukaha Military Reserve.**

## Rapid 'Ōhi'a Death Resistance: Assessing Carbohydrates and Chemical Defenses



Asa McCurdy uninstalling trail cameras at the end of project 1 that were used to capture flowering of 'ōhi'a lehua trees.

### INVESTIGATORS

**Asa McCurdy**, PhD Graduate Student, Department of Forestry and Natural Resources, Purdue University

**Simon Landhäusser**, Professor, Department of Renewable Resources, University of Alberta

**Morgan Furze**, co-PI, Assistant Professor, Departments of Botany and Plant Pathology / Forestry and Natural Resources, Purdue University

**Amy Trowbridge**, co-PI, Assistant Professor, Department of Forest and Wildlife Ecology, University of Wisconsin-Madison

### BACKGROUND / JUSTIFICATION

Invasive pathogens are an increasing threat to forests worldwide, reducing forest productivity and causing extensive tree mortality. To better predict pathogen establishment, spread, and disease impacts on forest ecosystem function, fundamental knowledge of host tree physiology and the pathogen-induced stress response are critical. Nonstructural carbohydrates (carbohydrates, i.e., sugars and starch produced through photosynthesis) are central to tree function and support critical metabolic processes, making them essential to survival. Further, they provide

the energy and building blocks to fuel secondary metabolic pathways in trees, the products of which play important roles in defending against destructive pathogens. Despite the potential for the relationship between carbohydrate storage and defense production to influence tree resistance, the linked dynamics in the presence and absence of a pathogen remain relatively unexplored. In Hawaiian forests, a novel destructive fungal pathogen, Rapid 'Ōhi'a Death (ROD; *Ceratocystis* spp.), threatens the keystone tree species *Metrosideros polymorpha* Gaud. ('ōhi'a lehua). To protect these native forests, there is an urgent need to identify resistant trees and the mechanisms responsible for enhanced survival. Our highly collaborative project is designed to merge basic and applied science to help meet that need. We integrate two different aspects of a plant's biotic stress response that have often been historically examined in isolation—carbohydrates and the pathogen-induced structural and chemical defense response—to develop a potentially new metric to identify how a tree's carbon reserves may buffer against biotic stress.

### PROJECT OBJECTIVES

We are coupling field and greenhouse-based experiments to quantify the relationship between carbohydrates and the structural and chemical defense response to ROD infection to improve our mechanistic insights into tree resistance as well as our ability to predict 'ōhi'a survival at the individual and population levels.

In project 1, we seek to characterize how carbohydrates and chemical defenses change across tree organs throughout the year in mature 'ōhi'a trees under business-



Setting up 'ōhi'a lehua for the greenhouse experiment in project 4.



Flowering 'ōhi'a lehua.

as-usual conditions without ROD infection. This will allow us to identify the times of year when 'ōhi'a trees may be more vulnerable to ROD infection.

In project 2, we will simulate pathogen infection in mature 'ōhi'a trees using the hormone methyl jasmonate to determine the induced defense response and its role in providing resistance to ROD infection.

In project 3, we will conduct a greenhouse experiment to determine how different 'ōhi'a varieties respond to drought, an abiotic factor that may compound ROD infection. This will also help identify varieties that are drought resistant for reforestation efforts.

In project 4, we will conduct a greenhouse experiment to assess the response of carbohydrates, chemical defenses (phenolics), and structural defenses (tyloses formation) to ROD infection in resistant and susceptible 'ōhi'a varieties.

#### **APPROACH**

- For project 1, we conducted an observational field-based study from 2024-2025 by repeatedly sampling healthy 'ōhi'a trees for carbohydrates and chemical defenses on a bi-monthly basis. We collected leaves, branches, stem xylem, stem phloem, and roots from each tree (n=9) for carbohydrates and total

phenolics. We also monitored flowering via trail cameras and quantified stem growth via point dendrometers.

- For project 2, we performed a pilot study in February 2025 to determine the dosage and duration of methyl jasmonate application required to elicit an initial chemical and structural defense response.
- For project 3, we performed a greenhouse drought experiment in Summer 2025 to assess the physiological responses of two 'ōhi'a varieties (incana and glaberrima) and koa to water stress. We characterized time to stomatal closure and dynamics of the hormone abscisic acid. The optical vulnerability method was also used to determine stem xylem vulnerability to drought-induced embolism.
- For project 4, we began a ROD inoculation experiment on one susceptible and one resistant 'ōhi'a variety in February 2026. We are quantifying the carbohydrate and chemical defense response over time.

### KEY FINDINGS / ACCOMPLISHMENTS

- For projects 1 and 3, data analyses are underway to identify the key findings from our work. We are excited to share these results with you next year!


### FUTURE PLANS

- For project 2, we are analyzing the preliminary data from the pilot study to plan a field-based experiment in which methyl jasmonate will be applied to mature 'ōhi'a trees and the structural (tyloses formation) and chemical defense response will be quantified over time.

- For project 4, the greenhouse team is collecting 'ōhi'a tissue samples over the course of three months following ROD inoculation and these samples will be analyzed in Spring 2026 to determine the timing of tyloses formation and chemical defense dynamics in response to ROD infection.
- We are beginning to explore the investment in nectar production of mature 'ōhi'a trees.

### PARTNERS COLLABORATORS

- Hāloa 'Āina
- Duncan Smith (University of Wisconsin Madison)
- Emmeline Seest, Mark Crooks, Faith McDevitt, Tawn Speetjens, Douglass Jacobs (Purdue University)
- Marc Hughes, Christian Giardina (Institute of Pacific Islands Forestry, USDA Forest Service)
- Ryan Belcher, Nainoa Goo, Kenjo Pollmann (Akaka Foundation for Tropical Forests)



Tropical HTIRC

## **RESEARCH PUBLICATIONS IN 2025**

68— The In Vitro and In Vivo Fungal Volatile Organic Compounds Associated with Rapid 'Ōhi'a Death and the Response of Xyleborine Ambrosia Beetles to those Compounds

# The *In Vitro* and *In Vivo* Fungal Volatile Organic Compounds Associated with Rapid 'Ōhi'a Death and the Response of Xyleborine Ambrosia Beetles to those Compounds

Journal of Chemical Ecology (2025) 51:59  
<https://doi.org/10.1007/s10886-025-01606-1>

## RESEARCH



## The *In Vitro* and *In Vivo* Fungal Volatile Organic Compounds Associated with Rapid 'Ōhi'a Death and the Response of Xyleborine Ambrosia Beetles to those Compounds

Kylle Roy<sup>1,2</sup> · Eva Brill<sup>3</sup> · Dan Mikros<sup>4</sup> · Kelsey Tobin<sup>2</sup> · Jennifer Juzwik<sup>5</sup> · Brandon Mcnellis<sup>6</sup> · Douglass Jacobs<sup>2</sup> · Lisa Keith<sup>3</sup> · Dong H. Cha<sup>3</sup> · Matthew Ginzel<sup>2,7</sup>

Received: 30 January 2025 / Revised: 2 May 2025 / Accepted: 5 May 2025

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### Abstract

Rapid 'ōhi'a death (ROD), caused by fungal pathogens *Ceratocystis lukuohia* and *Ceratocystis huliohia*, is devastating 'ōhi'a (*Metrosideros polymorpha*), the keystone forest tree species of Hawai'i. Ceratocystidaceae fungi produce fruity volatile organic compounds (VOCs) and sticky sexual spores, suggesting they are adapted for insect dispersal. In this study, we explored chemical signaling of the causal agents of the ROD disease complex and how those VOCs mediate ambrosia beetle (Coleoptera: Curculionidae: Scolytidae)-plant interactions. Four species of ambrosia beetles (*Xyleborinus saxesenii*, *Xyleborus affinis*, *Xyleborus ferrugineus*, and *Xyleborus perforans*) are direct vectors of the ROD pathogens in addition to spreading the fungi indirectly through contaminated frass. To further understand the extent to which semiochemicals mediate relationships between agents of the ROD pathosystem, we identified fungal VOCs both *in vitro* and *in vivo*. We then tested behavioral responses of the ROD-associated ambrosia beetles to the odor of the fungi using still-air olfactometer assays. Similar fruity and banana-like fusel alcohols and acetates were produced by *C. lukuohia* and *C. huliohia* in culture and when colonizing 'ōhi'a seedlings, which could be detected one-day post-inoculation, nine days before symptom onset. *X. affinis* was attracted to both *C. lukuohia* and *C. huliohia*, while *X. ferrugineus* positively responded to *C. lukuohia* and *X. perforans* to *C. huliohia*. These findings suggest a semiochemical association and potential support for the hypothesis of coevolutionary history between *Ceratocystis* fungi and insects.

## Extension / Outreach

Tropical HTIRC aims to ensure that research results are used by landowners, forest managers, and the scientific community. We collaborate directly with Tropical HTIRC to design research projects to meet their management needs. Our extension / outreach program consists of regular field days, workshops, symposia, and development of publications relevant to managers.



Field day at Kealakekua Mountain Reserve with Hiki Ola and the Kua O Ka Lā Public Charter School. Photo: Keli'ikanoe Mahi.

# State of Hawai'i Tree Nursery Assessment and Workshop Training

## INVESTIGATORS

**Owen Burney**, Professor, New Mexico State University

**Emily Senegal-Thyroff**, Senior Researcher and Extension Specialist, Department of Forestry and Natural Resources, Purdue University

**Tawn Speetjens**, PhD Graduate Student, Department of Forestry and Natural Resources, Purdue University

**Jeremiah Pinto**, Tribal Nursery Specialist, USDA Forest Service, National Center for Reforestation, Nurseries, and Genetics Resources

**Douglass Jacobs**, Fred M. van Eck Professor, Department of Forestry and Natural Resources, Purdue University

## BACKGROUND / JUSTIFICATION

Reforestation and forest restoration are integral parts of the State of Hawai'i's long-term management goals on DOFAW Forest Reserves and private lands. To help meet these goals, a steady supply of high quality, nursery-grown seedlings is necessary. Additionally, recent trends indicate that there is an increasing need for seedlings to support reforestation activities on state lands and with other landowners. This suggests a possible need to expand the state seedling nursery production capacity in quantity and quality to help meet the demands of these diverse reforestation projects. Prior to implementing such an expansion, however, a statewide survey may help to develop

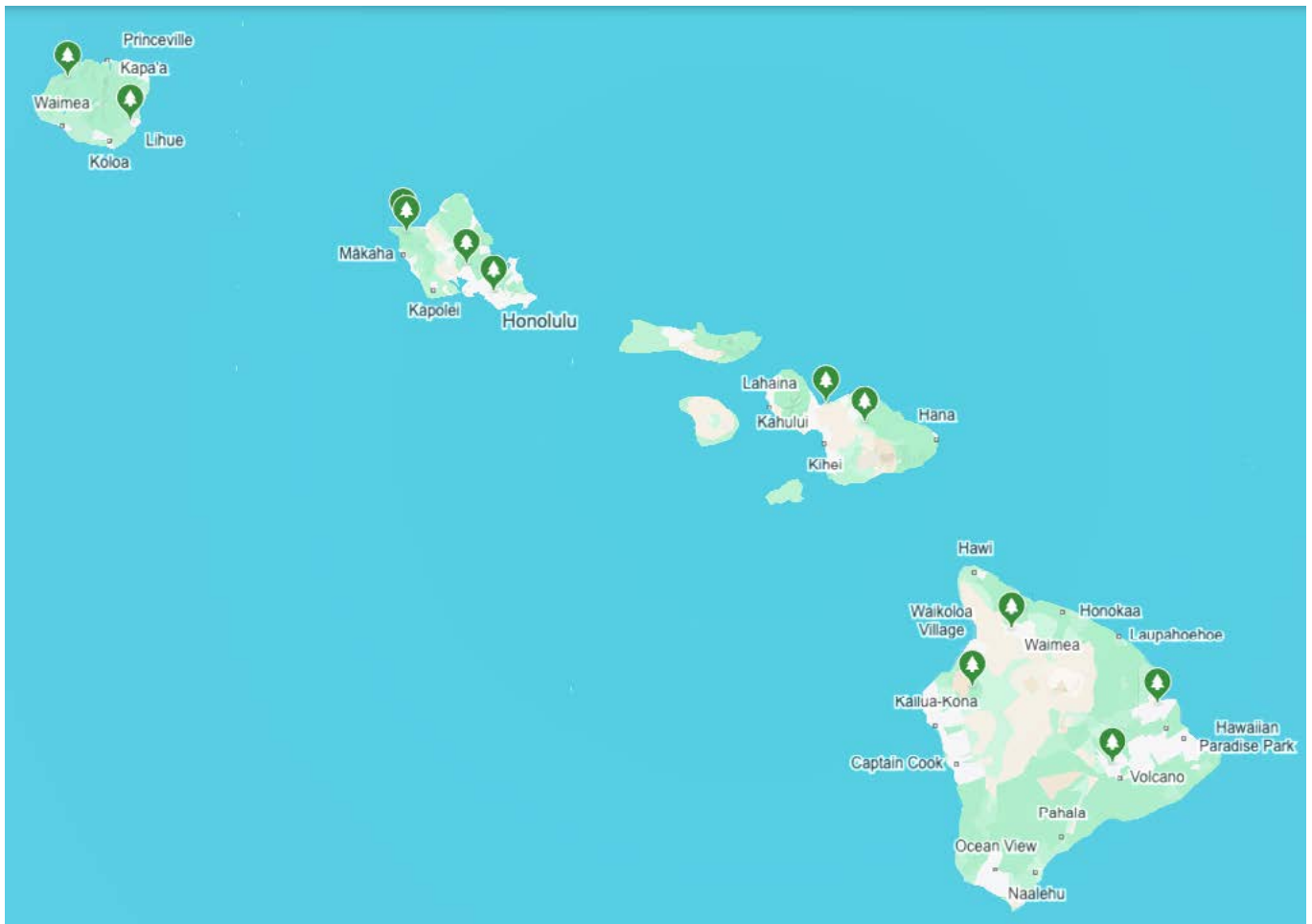
a concerted set of recommendations for if and how such an expansion might be implemented.

## PROJECT OBJECTIVES

Conduct a statewide survey, analysis, diagnosis, action plan and monitoring for the seedling production nurseries operated or managed by the State of Hawai'i with a vision toward potentially expanding the seedling production capacity in quantity and quality to meet future demand. As part of assessing the need for expansion, we also developed an estimate of reforestation projections and likely species targets for the next decade in the State of Hawai'i. We conducted this project with the direct collaboration of Division of Forestry and Wildlife (DOFAW) nurseries and staff, as well as external partners. Following the nursery assessment, we designed and conducted a series of training workshops for DOFAW nursery staff.

## APPROACH

- **Phase 1:** Research, visit, and evaluate the state-managed nurseries to understand their current production capacity (including labor, equipment, and facilities), species grown, seed information (collection, processing, and storage), propagation methods, quality control, sanitation protocols, standardization of procedures, revenue, and interactions with customers. This first phase provided a snapshot of current capacity and of recommendations for harmonizing and improving operating procedures at all state-managed nurseries.



Map of the 12 seedling production nurseries operated by DOFAW.

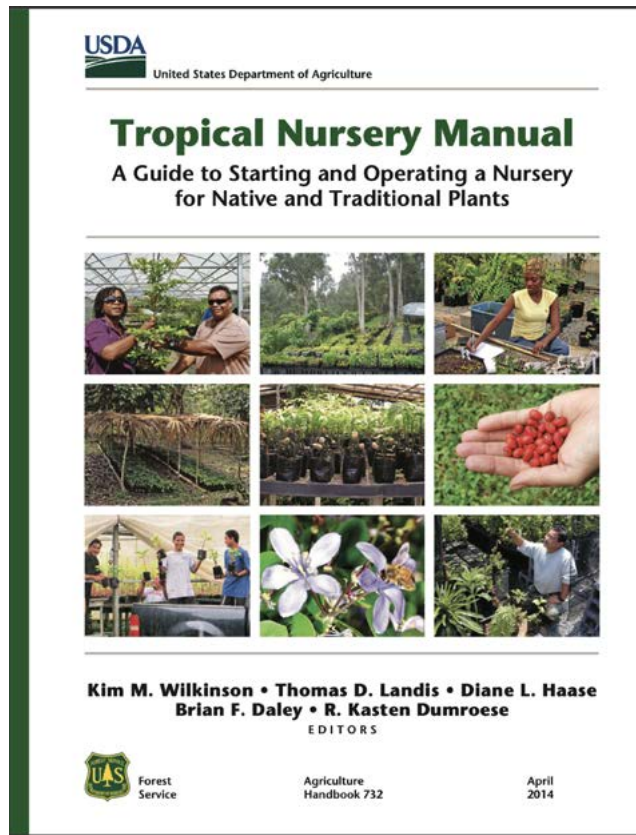
- **Phase 2:** Based on the findings in Phase 1, develop a series of recommendations to improve and potentially expand production at the various state nurseries, while ensuring quality. This phase considered the demand for seedlings, species mix, and seed sources, and created a forecast for tree seedlings over the next 10 years.
- **Phase 3:** Design and implement an initial training, learning, and inspection system for State of Hawai'i DOFAW nurseries and staff.

### KEY FINDINGS / ACCOMPLISHMENTS

- As previously reported, we visited and

surveyed each of the 12 nurseries operated or managed by the State of Hawai'i across four islands:

- Hawai'i (Hilo, Volcano, Waimea, Pu'uwa'awa'a)
- Kaua'i (Pua Loke, Kōke'e)
- Maui (Kahului, Olinda)
- O'ahu (Makiki, Waimano, Dillingham, Pahole)
- We also collaborated with DOFAW staff to develop a list of 23 key internal (DOFAW) and external collaborators that are actively engaged in reforestation and restoration projects in Hawai'i to assess



The USDA Forest Service Tropical Nursery Manual, an important resource for Hawai'i nurseries.

their strategic visions about reforestation in the state, including current and future needs for seed and seedlings.

- We identified a strong trend of increasing need for more plant material than is currently being grown by state and private nurseries in Hawai'i. There was unanimous concern that Hawai'i is not prepared to meet future demand for native forest tree seedlings.
- Most of the current seedling demand in Hawai'i is met by private nurseries.
- Lack of seed protocol and procedure is a major limiting factor in expansion capacity for state and private nurseries in Hawai'i.
- In 2025, we submitted our final report to DOFAW recommending: 1) a need for

strategic consolidation of infrastructure, staffing, and supplies in state-managed nurseries; 2) development of a new statewide "Nursery Director" position to help coordinate nursery operations and implement training programs; 3) strategically restructuring and enhancing the statewide nursery system using an appropriate combination of highly controlled greenhouses, semi-controlled, and/or minimally-controlled environments; and 4) develop a robust seed collection and banking program. We held three forums with DOFAW administrators and staff during 2025 to present and discuss our results and recommendations from the Phase 2 report.

- In 2025, we also assembled a team and



Nursery training workshop for State of Hawai'i DOFAW nursery staff in Volcano, HI during February 2025.



Hawai'i native plant nursery.

held two training workshops for State of Hawai'i DOFAW nursery staff (Volcano, HI and online). These workshops covered: water quality, irrigation, fertilization, crop planning, and crop hardening. We distributed copies of USDA Forest Service's Tropical Nursery Manual to all nursery staff. To complement these workshops, we also established a protocol for irrigation water sampling at all DOFAW nurseries, arranged for lab analyses of the samples, and provided each nursery with lab results and general water quality reference guidelines. Dr. Jeremiah Pinto (Tribal Nursery Specialist, USDA Forest Service) and Tawn Speetjens (PhD Graduate Student, Purdue University and past manager of Hāloa 'Āina nursery) contributed significantly to these workshops and the interpretation of water quality results.

### **FUTURE PLANS**

- An important outcome from this project was that DOFAW acted upon our recommendation to create a new "Statewide Horticulture Coordinator" position and we expect that this position will be filled in 2026.
- Although this project ended in 2025, we hope to assist with the initial improvement and expansion efforts of State of Hawai'i DOFAW nurseries by serving as facilitators for discussions within DOFAW and working with the new Statewide Horticulture Coordinator to develop / implement a training plan.

### **PARTNERS / COLLABORATORS**

Hawai'i Department of Land and Natural Resources (DLNR) Division of Forestry and Wildlife (DOFAW).

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## Forest Nursery Training in American Samoa



J.B. Friday teaching ASCC how to plan watering based on pot weight.

### INVESTIGATORS

**JB Friday**, Extension Forester, Department of Natural Resources and Environmental Management, University of Hawai'i at Mānoa

**Ashley McGuigan**, Assistant Extension Specialist, Department of Natural Resources and Environmental Management, University of Hawai'i at Mānoa

### BACKGROUND / JUSTIFICATION

American Samoa is a small, green, and rugged island in the South Pacific. Because it has been an American territory since 1900, American Samoa Community College

(ASCC) participates in the same USDA land grant extension and research programs as mainland US states, and the USDA Forest Service Reforestation, Nurseries, and Genetic Resources program (RNGR) also supports the forestry nursery there. The island is mostly forested, with both native forests and widespread agroforests producing coconuts, bananas, breadfruit, and other fruit and timber tree crops. The territorial forestry program is run by the ASCC forestry land grant program and focuses on supporting forest and agroforest landowners and local communities with both technical assistance



**Tree planting demonstration in a local agroforest.**

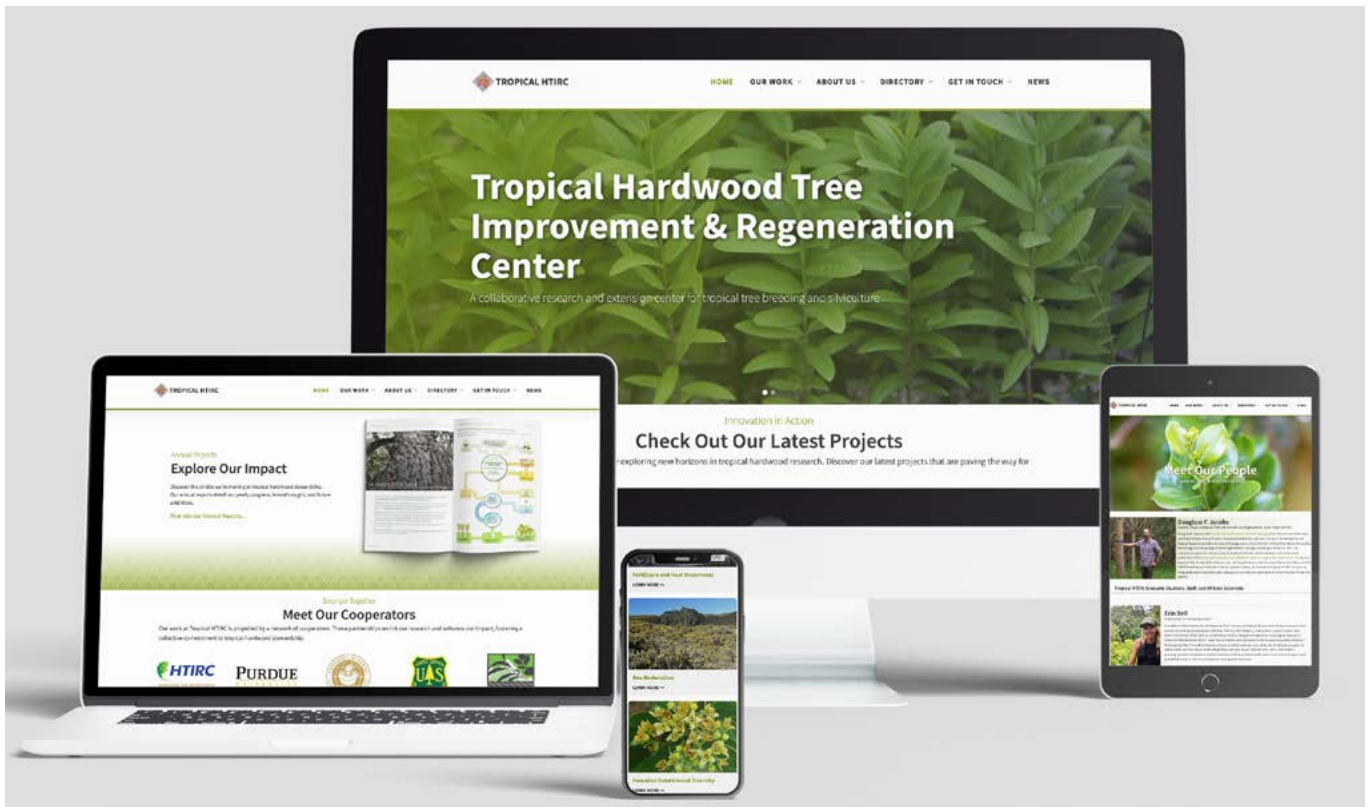
and forestry seedlings. In February 2026, University of Hawai'i Extension Forester J. B. Friday and Assistant Professor of climate, agroforestry, and nutrition Ashley McGuigan ran a 3-day workshop on forestry and agroforestry tree production hosted by Territorial Forester DJ Sene and the American Samoa Community College. Participants included not only staff from the forestry and horticulture programs at the college but also staff from the Territorial government, the National Park of American Samoa, and the USDA Natural Resources Conservation Service.

The current ASCC forestry nursery is being rebuilt and upgraded after a storm

destroyed the structure. Based on pre-workshop consultations with the forestry staff, workshop presentations included discussion of nursery design, irrigation systems, options for growing media, and methods to dry and store seeds, alongside lessons on forestry containers, management of pests and diseases, and other topics. Because locally produced cocopeat could partially replace the expensive imported potting soil the nursery now uses, ASCC Horticulturist Ian Gurr gave a demonstration of how to make cocopeat out of dry coconut husks using a mill that his program had purchased. ASCC Entomologist Mark Schmaedick gave an informative lecture on

different nursery pests and diseases found in Samoa and gave a demonstration of how to test for the presence of little fire ants (*Wasmania auropunctata*). The workshop also included visits to a local agroforest where participants planted native timber trees into a coconut-dominated forest. The overall emphasis was on improving plant quality and using the Target Tree concept to help produce seedlings that will thrive where they are planted. Each participant received a copy of the Forest Service Tropical Nursery Manual and a 10x hand lens to help them identify pests and disease. Participants ended the workshop by developing a list of practices they plan to implement to improve production in their own nurseries. The workshop also resulted in improved links between University of Hawai'i staff, USDA Forest Service staff, and agriculture and natural resource professionals in American Samoa.

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We continue to communicate new developments with our projects, staffing, and publications through our re-designed Tropical HTIRC website – where you can also access our 2022-2026 strategic plan and annual reports.

# Mahalo to Our Partners!





## MISSION

The Tropical HTIRC mission is to advance the science and application of tree improvement, management, and protection to improve tropical hardwood forests, with emphasis on the Hawaiian Islands.



Mahalo to our photography contributors: J.B. Friday & others  
Design & Layout by: 'Ākolea Visual

[WWW.TROPHITIRC.ORG](http://WWW.TROPHITIRC.ORG)



**Tropical Hardwood Tree Improvement  
& Regeneration Center**