REGENERATION OF ʻILIAHI (*SANTALUM PANICULATUM*): HEMIPARASITIC RELATIONSHIPS IN HAWAIIAN TROPICAL DRY FORESTS

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Dedicated to family, friends, and forests, who inspire me and encourage my curiosity and love of nature and science.

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Abstract

Santalum (sandalwood) species, including the endemic Hawaiian species known as ʻiliahi, are ecologically distinct as root hemiparasitic, evergreen trees requiring suitable hosts for long-term resource acquisition. Restoration and sustainable forestry of ʻiliahi at degraded sites, including threatened tropical dry forests, therefore, require supporting host plant populations. In this Ph.D. dissertation, I aimed to improve the survival and establishment of planted ʻiliahi, *Santalum paniculatum*, seedlings by better understanding the hemiparasitic relationship between ʻiliahi and native tropical dry forest host species. Three projects were implemented to address this overarching goal with the intention of integrating results into current stewardship strategies. The first project compared host dynamics for ʻiliahi paired with different species at varying planting distances. In June 2019, 360 one-year-old container ʻiliahi seedlings were planted in a completely randomized experimental design. ʻIliahi seedlings were randomly assigned one of nine treatments: ʻiliahi with (1) no host (control), (2) koa at <0.2 m, (3) koa at 0.5 m, (4) koa at 1.0 m, (5) koa at 2.0 m, (6) ʻaʻaliʻi at <0.2 m, (7) ʻaʻaliʻi at 0.5 m, (8) ʻaʻaliʻi at 1.0 m, and (9) ʻaʻaliʻi at 2.0 m. After three years, ʻiliahi grew more and had greater foliar nitrogen concentration when paired with koa (*Acacia koa*) at a close distance than with ʻaʻaliʻi (*Dodonaea viscosa*) at any distance or the control with no host. In the second project, I assessed potential tradeoffs between competition and parasitism of ʻiliahi planted under an established koa host canopy. In August 2020, 324 nine-month-old container ʻiliahi seedlings were planted under the canopy of ten-year-old koa trees. Canopy openness above each ʻiliahi seedling varied throughout the planting site with a range of 8.8 % to 90.1 %. ʻIliahi seedling height and diameter were greater with larger canopy openness, and physiological measurements also increased with greater canopy openness. In the third project, I examined the transfer of resources between ʻiliahi and its host. In April 2021, 2-pot and 3-pot experimental units were established with the roots of ʻiliahi and koa split between containers. The 2-pot and 3-pot units allowed the segregation of roots to examine root exudate transfer and haustoria transfer between ʻiliahi and koa. In April 2022, ^{15}N and ^{13}C stable isotope tracers were applied to specific units and species. We detected ^{15}N root exudate transfer and ^{15}N transfer from the host koa to the hemiparasite ʻiliahi. One- and two-way transfer of ^{13}C were detected in the roots; however, we are cautious as to the biological significance of this result. These results contribute to our understanding of hemiparasitic plants and improve efforts to restore native Hawaiian dry forests, form a sustainable ʻiliahi silviculture basis, and contribute to sustaining functionally compatible and abundant forests.

Chapter 1 - Introduction

1.1 Plant Relationships and Hemiparasitism

Forests are complex ecosystems with many interacting components and a range of plant relationships. These interactions and relationships play a crucial role in plant community dynamics. Interactions can be positive, such as facilitation (Michalet and Pugnaire, 2016). Alternatively, interactions can be negative, such as competition and parasitism (Matías and Jump, 2012; Těšitel, 2016). While competition can negatively affect both plants, parasitism adds an aspect to negative biotic interaction, where the host is proportionally more negatively affected. Obligate parasites entirely depend on their host, whereas hemiparasites can be less dependent given their ability to photosynthesize (Bell and Adams, 2011; Matthies, 2017; Těšitel et al., 2010). Plant resource acquisition is also affected by how resources are cycled and transferred within an ecosystem (McNickle and Dybzinski, 2013; Schwinning and Kelly, 2013).

Approximately 1% of all angiosperms are parasitic, and most are root hemiparasites (Heide-Jørgensen, 2013; Matthies, 2017). Despite parasitism evolving independently numerous times in angiosperms, resources are consistently transferred directly from the host to the parasite via specialized structures called haustoria (Figure 1.1; Barkman et al., 2007). Root hemiparasites have haustoria connecting to hosts' roots to acquire resources (Barkman et al., 2007; Bell and Adams, 2011). In addition to acquiring resources for hosts, host plants may also be competitors for available resources (Das, 2021a).

Specific anatomical connections determine how resource transfer via haustoria occurs (Těšitel, 2016). For example, parasitic connections can be phloem and xylem transfer or limited to xylem transfer with selective cross membrane exchanges as opposed to mass flow via vascular continuity (Ouyang et al., 2015; Pate, 2001; Tennakoon and Cameron, 2006). Additionally, haustoria biomass was positively correlated with growth, indicating that the total surface area of haustoria appears to be influential in the transfer of resources (Lu et al., 2014).

Figure 1.1 ʻIliahi, *Santalum paniculatum*, haustoria connecting to koa, *Acacia koa*. Photos: Emily Thyroff.

1.2 Santalaceae Family

Most hemiparasitic, woody angiosperms belong to the Oleaceae and Santalaceae families (Veenendaal et al., 1996). Within the Santalaceae family are the *Santalum* spp., a prominent example of root hemiparasite trees ranging from India, Australia, and islands throughout the Pacific Ocean (Teixeira da Silva et al., 2016; Wagner et al., 1999). Six of the 24 presently recognized *Santalum* spp. and several varieties are endemic to the Hawaiian Islands (Harbaugh et al., 2010), locally known as ʻiliahi (Figure 1.2). Internationally, *Santalum* spp. have been, and still are, coveted economically and culturally as sources of sandalwood oil and wood (Thomson et al., 2011). Due to this resource value, severe exploitation of *Santalum* spp. occurred throughout its range (Teixeira da Silva et al., 2016), including with ʻiliahi in Hawaiʻi (St. John, 1947). Hemiparasitism in Hawaiian forests has been minimally studied, yet hemiparasitism was likely a common forest relationship based on large estimated former ranges of endemic ʻiliahi (Price et al., 2012).

Figure 1.2 (Left) Five-month-old ʻiliahi seedlings, *Santalum haleakalae*, endemic to Maui. (Right) Flowering ʻiliahi, *Santalum freycinetianum*, endemic to Oʻahu. Photos: Emily Thyroff.

ʻIliahi was once abundant across Hawaiian forest types, including tropical dry forests, but now the species are rare due to intensive overharvesting (Merlin et al., 2006; St. John, 1947). Populations of a few species have increased since the decline of overexploitation around 1830 (St. John, 1947); however, the species have not regained their former range due to the conversion of suitable habitat to agriculture, grazing, and the spread of feral and wild ungulates such as cattle and sheep (Merlin et al., 2006). Only one ʻiliahi species, *Santalum paniculatum* (Hook. & Arn.), endemic to Hawaiʻi Island, is currently harvested in limited quantities, primarily for oil (Figure 1.3; Braun et al., 2014). Due to its value and relative scarcity, there is considerable interest in reestablishing ʻiliahi and learning more about its biology and ecology for restoration and for potential sustainable silvicultural systems (Nageswara-Rao et al., 2012). The hemiparasitic nature of ʻiliahi makes reestablishing and supporting other native species essential to serve as hosts within a forest context.

Figure 1.3 Mature ‘iliahi, *Santalum paniculatum*, in protected forest areas on Hawai‘i Island. Photos: Emily Thyroff.

1.3 Tropical Dry Forests and Regeneration Status

Hawaiian tropical dry forests are one forest type where ‘iliahi are located (Wagner et al., 1999) and where ‘iliahi were once abundant. Tropical dry forests have distinct dry periods, lower annual rainfall, and are globally among the most threatened biome (Pau et al., 2009). In contrast to wet tropical forests, dry tropical forests are typically defined more by limiting abiotic factors (e.g., water availability and extreme temperature) rather than competition, which can make reforestation and restoration more challenging (Cabin et al., 2002). Within the context of native Hawaiian dry forest species, ‘iliahi is reported to have intermediate carbon gain and lower leaf water potential (Sandquist and Cordell, 2007). Sustaining lower leaf water potential may maintain a favorable gradient to encourage the flow of resources from the host to ‘iliahi (Radomiljac et al., 1999b; Sandquist and Cordell, 2007).

The threatened status of tropical dry forests is partially due to restrictive abiotic factors and preferential use for other activities, such as grazing, agriculture, and residential and commercial developments due to amicable climates (Cordell et al., 2002; Griscom and Ashton, 2011). In

Hawaiʻi, tropical dry forests were regularly cleared for agricultural uses that often have since been abandoned. Compounding the exploitation legacy of depleted ʻiliahi populations across the Hawaiian Islands, Hawaiian tropical dry forests, for example, have been reduced by 90 % with dispersed parcels of remaining dry forest (Medeiros et al., 2014).

Natural regeneration of many native Hawaiian trees, including ʻiliahi, is limited by several factors. Examples include seed predation, limited germination, herbivory, competition from invasive plants, decline or loss of pollinators, and greatly increased frequency and spread of wildfire (Friday et al., 2015; Pau et al., 2009; Thaxton et al., 2012). While ʻiliahi can prolifically coppice and root sucker, particularly after harvest, this only leads to a localized increase in abundance (Speetjens et al., 2021). Artificial regeneration (i.e., tree planting) of ʻiliahi is necessary to restore populations of ʻiliahi across their natural range. Given the hemiparasitic nature of ʻiliahi and limitations to natural regeneration of native species, success is likely dependent upon the effective establishment of both the hemiparasite and hosts (Das, 2021a). Due to the hemiparasitic relationship and history of dry forest conversion to other land uses reducing suitable habitat, ʻiliahi plantings often also necessitates the planting of hosts.

1.4 Hosts and Spacing

Host plants of parasitic angiosperms are diverse and range from herbaceous plants to shrubs and trees. Hemiparasite plants may successfully parasitize many species without specificity or be restricted to a few options for host species (Bell and Adams, 2011). Host plants vary in their ability to provide necessary resources; therefore, host suitability for various hemiparasitic species has been studied (Irving and Cameron, 2009; Matthies, 2017; Nge et al., 2019). Poor hosts may not be able to acquire and transfer the resources parasites need, or poor hosts may have defense mechanisms (Cameron and Seel, 2007). Hosts are classified as suitable when the hemiparasite's survival and growth is greater than when paired with another potential host or growing without a host (Brand, 2009; Nge et al., 2019; Ouyang et al., 2015; Teixeira da Silva et al., 2016). High net mineral content gain is another sign of suitable hosts compared to net losses or small gains (Radomiljac et al., 1999c; Struthers et al., 1986).

A wide diversity of plant species, native and non-native to Hawaiian dry forests, may be effective candidates to serve as hosts to ʻiliahi. There is a strong impetus to use native species as hosts for ʻiliahi as part of native forest restoration, for biocultural restoration, and the economic value of certain native tree species (Friday et al., 2022). There is no comprehensive list, however, of native species identified as suitable or preferred hosts for ‘iliahi. ‘A‘ali‘i (*Dodonaea viscosa* Jacq.) and koa (*Acacia koa* A. Gray) are two native species that are currently regularly paired with ‘iliahi in reforestation projects (Figure 1.4).

‘A‘ali‘i is common in restoration projects given its high survival, growth, and ability to facilitate recruitment of other native plants (Yelenik et al., 2015). ‘A‘ali‘i has been identified as a potential suitable host not only for ‘iliahi, but also for other *Santalum* spp. (Soosairaj et al., 2005; Thomson et al., 2011). Nitrogen-fixing woody plants, such as *Acacia* spp., have also been identified as suitable and preferential hosts for many *Santalum* spp. given the importance of acquiring nitrogen (Lu et al., 2014; Nge et al., 2019; Radomiljac et al., 1999; Veillon and Jaffré, 1995; Woodall and Robinson, 2003). In Hawai‘i, koa is a common forest restoration species, given its relatively high survival as a planted seedling, fast early growth, presence across large rainfall gradients, and tolerance to many abiotic factors (Hamilton et al., 2021; Jacobs et al., 2020; Rose et al., 2020; Scowcroft et al., 2008).

Operationally once host species are identified, the combination of hosts, planting structure (e.g., planting grid, nucleation plantings, seedling density), and distances need to be determined. Many hosts can be paired with ‘iliahi, whether to resemble a native forest, in a mixed species plantation, or part of an agroforestry system (Das, 2021b; Goswami, 2014; Lu et al., 2020; Thomson, 2006; van Noordwijk et al., 2001). The distance between hemiparasite and host can vary and is an important consideration (Page et al., 2012) because distance may influence how quickly and how many parasitic connections form. Determining an effective spacing between ‘iliahi and its hosts is complicated because the size of the plants, resource requirements, growth rates, plant allometry, root expansion, and competitiveness will likely change over time, as observed with other *Santalum* spp. (Hamilton and Conrad, 1990; Thomson et al., 2011). While establishing haustoria and resource transfer is likely critical for survival and growth of ‘iliahi, there must be balance between parasitic resource transfer and competition both above- and below-ground to ensure the success of both parasite and host as part of a restoration strategy.

Figure 1.4 Examples of ‘iliahi, *Santalum paniculatum*, growing with ‘a‘ali‘i, *Dodonaea viscosa*, and koa, *Acacia koa*, nearby. Koa is the tallest in both photos, ‘iliahi has pink liko (new growth) and ‘a‘ali‘i has lanceolate leaves. Photos: Emily Thyroff.

1.5 Underplanting

Underplanting could be an approach to study tradeoffs between the benefits of resource transfer from the host root system and competition with the host. Spacing from hosts and competition from hosts, especially for light, (Matthies, 1995), are important planting and underplanting considerations. Underplanting involves planting seedlings under an existing forest canopy and is an effective solution for certain reforestation and restoration scenarios (Paquette et al., 2006). Limiting factors that underplanting may facilitate in overcoming include species that are later successional, shade-tolerant, or susceptible to browse (Dey et al., 2012; Maltoni et al., 2019; Rose et al., 2019; Thyroff et al., 2019). Underplanting may be particularly beneficial for species with unique relationships with other plants, such as parasitic or hemiparasitic plants, because of their dependence on host root networks (Figure 1.5).

Figure 1.5 Ten-year-old koa, *Acacia koa*, stand with a range of canopy openness that ʻiliahi, Santalum paniculatum, was planted under. Photo: Emily Thyroff

Planting hemiparasites under established host plants, rather than planting hemiparasites and hosts simultaneously, may allow the hemiparasites to connect more quickly to an already established host root system. Although hemiparasites rely on hosts for water and nutrients, the hemiparasites are also competing with hosts, including for light (Matthies, 1995); thus, as with plant regeneration under varying overstory conditions more generally, there may be an optimal overstory condition and resulting light level (Rose et al., 2019; Soto et al., 2017). Furthermore, shade tolerance of underplanted hemiparasitic trees may be dynamic over time. While increased light often encourages the establishment of species in wet forests of Hawaiʻi, in dry forests, full sun may be detrimental due to increased temperatures and evapotranspirational demands (Cordell et al., 2002; Inman-Narahri et al., 2013).

Additionally, single-species reforestation or natural regeneration with only one or a few dominant species can be diversified by underplanting (Messier et al., 2022; Paquette et al., 2006). Due to koa's cultural significance, economic value, and ecological role koa is often one of the first species naturally established or outplanted at reforestation and restoration sites (Scowcroft and Yeh, 2013). Thus, many reforestation sites consist of planted or naturally regenerated, single-aged koa stands. Koa also has rapid early growth, the ability to overtop weeds, and relatively light canopy, which all aid in establishing a canopy for future native species regeneration (Funk and McDaniel, 2010; Pearson and Vitousek, 2001; Scowcroft et al., 2008; Scowcroft and Yeh, 2013).

1.6 Resource Transfer

Identifying what resources ʻiliahi extract from hosts can elucidate which native hosts and what kind of planting arrangement are more compatible, leading to increased ʻiliahi seedling survival and growth. As an essential plant macronutrient, nitrogen is an important resource hemiparasites acquire from hosts, sometimes comprising over half of the hemiparasite's total nitrogen (Tennakoon et al. 1997; Lu et al. 2014; Pageau et al. 2003, Jiang et al. 2004, Cameron and Seel 2007). Several other mineral nutrients (e.g., Ca, K, P, Na, Cu) seem to be exchanged between *Santalum* spp. and hosts when a hemiparasitic connection is made (Radomiljac et al., 1999c; Struthers et al., 1986). From literature on hemiparasite and other *Santalum* spp., water and nutrients are known to be acquired by *Santalum* spp. from its hosts, but since hemiparasites can photosynthesize, the role of host photosynthesis is unclear (Figure 1.6).

Figure 1.6 (Left) Three-pot experimental setup to elucidate what resources are transferred and the types of transfer occurring between a hemiparasite and host. (Right) Preparation for applying $^{13}CO_2$ stable isotope tracer. Photos: Emily Thyroff.

More often, resource transfer is thought to flow in one direction from host to hemiparasite; however, two-way transfer of nutrients from *S. album* (L.) to its hosts has been observed for both nitrogen and phosphorous (Lu et al., 2013; Rocha et al., 2015). There is even evidence that *Santalum* spp. self-parasitize, with haustoria attaching to its own root system (Radomiljac et al., 1999c; Lu et al., 2020). If two-way transfer occurs, then the hemiparasitic relationship should be viewed even more dynamically with the host potentially gaining resources from the hemiparasite. In addition to direct transfer via haustoria, resources can potentially also be directly transferred to the parasite via mycorrhizal connections (He et al., 2009; Li and Guan, 2008) or indirectly through leaf leachates, root exudates and associated soil redistribution processes (Coskun et al., 2017; Davies and Graves, 1998; Høgh-Jensen, 2006; Press, 1989). These transfer pathways apply to forest plant species generally and may be difficult to distinguish from the direct transfer via parasitic connections.

1.7 Justification and Objectives

During this time of ambitious calls for restoration and reforestation (e.g., the United Nations Decade of Ecosystem Restoration natural climate solutions, Bonn Challenge, and other incentive mechanisms), improving facets, techniques, and approaches of the reforestation pipeline is imperative to realize these goals (Aronson et al. 2020; Stanturf 2021), (Griscom et al. 2017) (Fargione et al. 2021) (Stanturf et al. 2019; Tedesco et al. 2022). To meet forest restoration and reforestation goals in absence of adequate natural forest regeneration (i.e., trees germinating and growing without intervention), artificial forest regeneration (i.e., the planting of seedlings or sowing of seeds) is often required. These terms are comparable to passive restoration and active restoration, and these passive and active efforts can complement and even augment each other (Scowcroft and Yeh, 2013). Regeneration and restoration efforts can also be aided by incorporating the Target Seedling Concept framework, including site-specific techniques and how limiting factors affect seedling survival (Dumroese et al., 2016).

Although six *Santalum* spp. are endemic to Hawai‘i (Harbaugh et al., 2010), scientific literature on *Santalum* spp. almost exclusively consists of focus on species outside of Hawai‘i including *S. album*, *S. acuminatum* (A.DC.), and *S. spicatum* ((R.Br.) A.DC.). The endemic diversity, unique Hawaiian biogeochemistry, and growing desire to regenerate ‘iliahi for ecological restoration and commercial forestry make it critical to better understand how hemiparasitism manifests in Hawaiian forests. Due to its scarcity and value, including ecological services and functions, there is significant local interest in restoring native Hawaiian tropical dry forests and, specifically, ʻiliahi regeneration. However, the biology and ecology of ʻiliahi, as well as the conditions for optimum tropical dry forest regeneration are not well understood. The hemiparasitic relationship, resource allocation between hemiparasite and host, management practices such as host suitability of various native species, and underplanting need to be further developed to successfully and efficiently regenerate ʻiliahi in the context of tropical dry forests. Additionally, in a review on parasitic plants, Těšitel (2016) posited that the uniqueness of parasitic plants makes it imperative to study specific species as unexpected natural processes may be revealed.

Through this Ph.D. dissertation research, my goal was to improve the survival and establishment of planted ʻiliahi seedlings. This dissertation contains three research projects to address this goal. In the first project (Chapter 2), I compared host dynamics for ʻiliahi with different hosts at different planting distances. The objective was to examine ʻiliahi seedling development when planted with two hosts at four distances to address host suitability and planting distance questions. In the second project (Chapter 3), I assessed tradeoffs between competition and parasitism of ʻiliahi planted under an established host canopy. The objective was to investigate ʻiliahi seedlings performance when planted under established koa canopies to address underplanting and canopy openness questions. In the third project (Chapter 4), I examined the transfer of resources between ʻiliahi and its host. The objective was to explore nitrogen and carbon transfer within the hemiparasitic relationship to address resource transfer questions. In Chapter 5, I synthesized key conclusions, and suggest future research directions.

A combination of field and nursery experiments at Kealakekua Mountain Reserve on Hawaiʻi Island were used in the three projects to address the research objectives. Kealakekua Mountain Reserve is an approximately 3,900-ha property of former agricultural land designated for tropical dry forest restoration and production forestry. The rainfall and temperature patterns for the property align with Hawaiian tropical dry forests (Appendix 1.1). For all three projects, morphological and physiological measurements were used to evaluate ʻiliahi and host suitability, underplanting dynamics, and hemiparasitic resource transfer. Throughout these research projects, Kanaka ʻŌiwi oli and kilo protocols were followed to ground my work in Hawaiian lifeways (Appendix 1.2).

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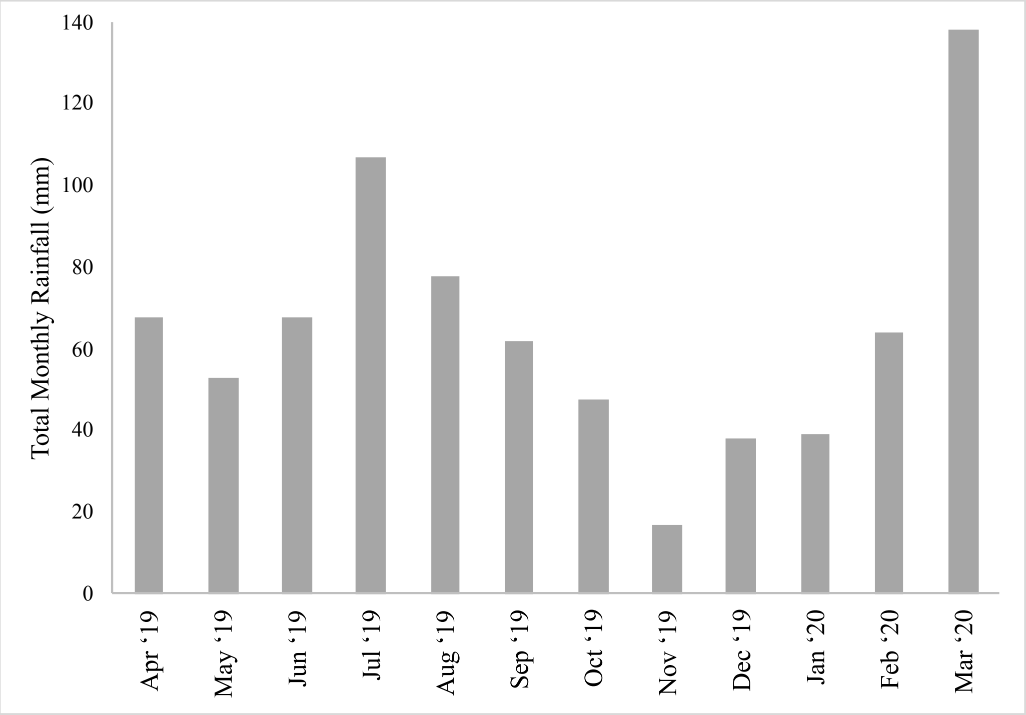
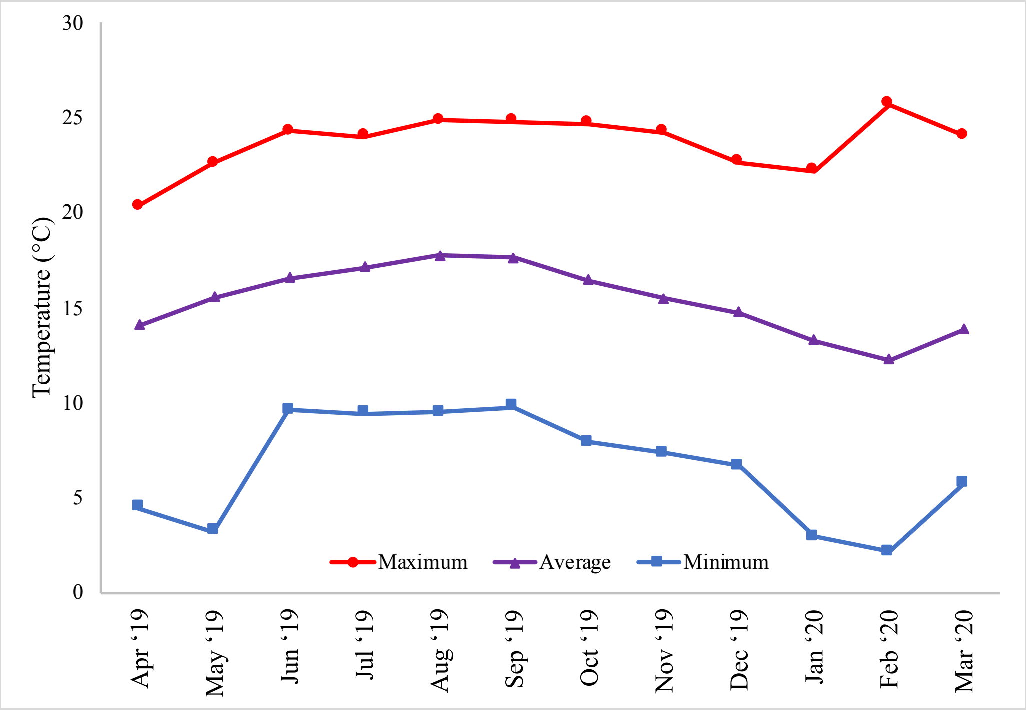
1.9 Appendix 1

Appendix 1.1 Weather station data from Kealakekua Mountain Reserve from April 2019 to March 2020 for total monthly rainfall (inches) and minimum, average, and maximum temperatures (°F). Approximately 740 mm of rainfall this year.





Appendix 1.2

Oli (Hawaiian chant) and kilo (careful observation) protocols were incorporated through my dissertation research. Below is the primary oli I gave to set intentions for the research, ask permission to enter spaces, and request guidance for the work. Kilo is defined as *to watch closely, spy, examine, look around, observe, forecast* in the Ulukau database (http://wehewehe.org/gsdl2.85/cgi-bin/hdict?l=haw). In addition to observations for protocoled data collection, additional observations or nā kilo were recorded and shared.

An Oli for CTAHR

Composed by Dr. Mehana Vaughan, originally for a group of undergraduate students conducting summer research on the island of Kauaʻi, this oli was shared with the College of Tropical Agriculture and Human Resources on the occasion of CTAHR's Silver Anniversary Awards Banquet on May 3, 2013.

The oli honors the communities and places in which we learn and work. Dr. Vaughan encourages its use to welcome, ask permission to enter, or request guidance for educational and research efforts, that they may bring pono for Hawaiʻiʻs ʻāina and people.

He aloha no ka ʻāina uluwehi
Wehi ke aloha o nā kūpuna e
He pūnāwai ʻike kau i ka hano
Hanohano wale o Hawai'i e
E hō mai ka maopopo pono
Pono mai uka a i kai e
Aloha e, Aloha e, Aloha e

Aloha for the abundant / verdant land
Adorned with aloha of the ancestors,
a spring of honored knowledge placed above
Just majestic is Hawaiʻi
Grant us understanding
That brings balance and thriving from mountains to sea
Aloha

Chapter 2 - Co-Planting of a Fast-Growing, Nitrogen-Fixing Host Tree Facilitates Regeneration of the Root Hemiparasitic ‘Iliahi (Hawaiian sandalwood)

2.1 Abstract

Root hemiparasitic trees can photosynthesize yet also acquire resources from host plants, which may benefit the long-term survival and growth of the hemiparasite. Experimental evaluation of planting distance between tree hemiparasites and their hosts can lead to biological insights and contribute to practitioner decision making. On an abandoned pasture site in a historically tropical dry forest in Hawai‘i, we studied the effects of two host species and four planting distances on the survival and growth of ‘iliahi (Hawaiian sandalwood, *Santalum paniculatum*), an endemic root hemiparasitic tree. Treatments included a control with no host or one of two native host species: ‘a‘ali‘i (*Dodonaea viscosa*), a fast-growing shrub, or koa (*Acacia koa*), a fast-growing nitrogen-fixing tree. ‘Iliahi and host seedlings were planted at a distance of <0.2, 0.5, 1.0, or 2.0 m from each other. After three years, survival of ‘iliahi seedlings was greatest for ‘iliahi paired with koa at 1.0 m (78 %) compared to the control with no host (53 %) and when paired with koa at 2.0 m (55 %). Ground line diameter and height of ‘iliahi seedlings were greater when paired with koa at closer distances than with ‘a‘ali‘i at any distance or the control with no host. Specifically, ‘iliahi growth was greatest when paired with koa at <0.2 m distance. Foliar nitrogen concentration was greater for ‘iliahi paired with koa at <0.2, 0.5, and 1.0 m distances, whereas the other foliar nutrient concentrations were typically greater for the ‘iliahi control with no host. For sites with few or no pre-established hosts, such as abandoned pastures, ‘iliahi growth can be greatly improved by co-planting in proximity to koa. As with other *Santalum* spp., early and abundant parasitic root connections with a host, especially a nitrogen-fixing host, are likely important for ‘iliahi establishment and early growth.

2.2 Introduction

Hemiparasitic plants are capable of photosynthesis yet also benefit from host plants by acquiring resources to ensure their long-term survival (Bell and Adams, 2011; Matthies, 2017). Root hemiparasites have specialized root structures called haustoria that connect hosts’ roots and transfer resources (Barkman et al., 2007; Bell and Adams, 2011). For example, nitrogen acquired from hosts can comprise large proportions of total nitrogen content in hemiparasites

(Cameron and Seel, 2007; Lu et al., 2014; Tennakoon et al., 1997). Host plants may also be competitors for available resources (Das, 2021a). Host suitability differs by species, as indicated by variable hemiparasitic performance (Irving and Cameron, 2009; Matthies, 2017; Nge et al., 2019). Hosts are classified as suitable when the hemiparasite experiences vigorous performance, i.e., greater survival, height and diameter, compared to growth with other hosts or growing without a host (Brand, 2009; Nge et al., 2019; Ouyang et al., 2015; Teixeira da Silva et al., 2016).

The *Santalum* genus (Santalaceae family), commonly referred to as sandalwoods, is a large group of root hemiparasitic trees and are part of ecosystems in Australia, India, and throughout the Pacific Ocean (Hamilton and Conrad, 1990; Teixeira da Silva et al., 2016; Wagner et al., 1999). Six of the 24 currently recognized *Santalum* spp. are endemic to the Hawaiian Islands (Harbaugh et al., 2010) and locally known as ʻiliahi. The aromatic heartwood imparts cultural and economic importance, which has led to the overexploitation of most *Santalum* spp. including ʻiliahi (St. John, 1947; Teixeira da Silva et al., 2016; Thomson et al., 2011). Natural regeneration of many native Hawaiian trees, including ʻiliahi, is limited by several factors. Examples include seed predation, low germination rates, herbivory, competition from invasive plants, decline or loss of pollinators, and greatly increased frequency and spread of wildfire (Friday et al., 2015; Pau et al., 2009; Thaxton et al., 2012). Artificial regeneration (e.g., tree planting) of ʻiliahi is necessary to restore populations of ʻiliahi across their natural range. Given the hemiparasitic nature of ʻiliahi and limitations to natural regeneration of native species, success is likely dependent upon the effective establishment of both the hemiparasite and hosts (Das, 2021a). Aspects of hemiparasite and host plant interactions have been researched for other *Santalum* spp. (Pullaiah et al., 2021; Teixeira da Silva et al., 2016), although distance between *Santalum* spp. and their hosts is lacking experimental insight.

A wide diversity of plant species co-occur with ʻiliahi in dry forests, potentially serving as hosts. Although, there is a strong impetus to incorporate native species into reforestation efforts in Hawaiʻi for many reasons, including biocultural restoration and economic value of native tree species (Friday et al., 2022). Nitrogen-fixing woody plants, such as *Acacia* spp., have been identified as suitable and preferential hosts for many *Santalum* spp. given the importance of acquiring nitrogen for seedling performance (Lu et al., 2014; Nge et al., 2019; Radomiljac et al., 1999; Veillon and Jaffré, 1995; Woodall and Robinson, 2003). In Hawaiʻi, an endemic nitrogen-fixing tree, koa (*Acacia koa* A. Gray), could be a preferred host species to ʻiliahi and by practitioners. Koa is a common forest restoration species, given its relatively high survival as a planted seedling, fast early growth, and ability to create a suitable understory for other native plants at maturity (Hamilton et al., 2021; Jacobs et al., 2020; Rose et al., 2020; Scowcroft et al., 2008). The native shrub ‘a‘ali‘i (*Dodonaea viscosa* Jacq.) has also been identified as a suitable host for *Santalum* spp. (Soosairaj et al., 2005; Thomson et al., 2011). ‘A‘ali‘i is common in Hawaiian dry forests and is planted in restoration projects, given its high survival, growth, and ability to facilitate recruitment of other native plants (Yelenik et al., 2015). Classified as a shrub or small tree, ‘a‘ali‘i could be a less competitive host than the even quicker growing koa. Lastly, many hosts can be paired with ‘iliahi simultaneously, whether to resemble a native forest, in a mixed species plantation, or part of an agroforestry system (Das, 2021b; Goswami, 2014; Lu et al., 2020; Thomson, 2006; van Noordwijk et al., 2001).

When planting hemiparasites with a host plant, the distance between species can vary and is an important consideration (Page et al., 2012). Few studies have experimentally examined host distance, and the effect of distance may depend on the host species (Keith et al., 2004). The distance between the hemiparasite and host may influence how quickly and how many parasitic connections form, and also influence the likelihood that competition for light may mitigate benefits of host connections (Keith et al., 2004). While establishing haustoria and resource transfer is likely important for long-term survival and growth of ‘iliahi, there must be balance between parasitic resource transfer and competition both above- and below-ground. Determining an effective spacing between ‘iliahi and its hosts is complicated because the size of the plants, resource requirements, and competitiveness will likely change over time, as observed with other *Santalum* spp. (Hamilton and Conrad, 1990; Thomson et al., 2011).

This study aimed to improve ‘iliahi (*Santalum paniculatum* (Hook. & Arn.), endemic to Hawai‘i Island) regeneration success through our understanding of ‘iliahi and host interactions. Specifically, survival and growth over three years were assessed when ‘iliahi was co-planted with native dry forest host species (‘a‘ali‘i or koa) at varying distances from each other. We hypothesized that ‘iliahi seedling performance would be greater when (i) paired with a host compared to no host, (ii) paired with a nitrogen-fixing tree versus when paired with a non-nitrogen-fixing shrub, and (iii) planted closer to the host (e.g., <0.2, 0.5 m).

2.3 Materials and Methods

2.3.1 Experimental Site

This experiment was conducted at Kealakekua Mountain Reserve, an approximately 3,900-ha property designated for tropical dry forest restoration and sustainable forestry where forest harvesting, pasture establishment, and grazing have inhibited natural regeneration of native forest species, including ʻiliahi. Non-native pasture grass (kikuyu, *Cenchrus clandestinus* Hochst. ex Chiov.) dominate the experimental site (19.4960° N, -155.80841° W). Kealakekua Mountain Reserve is within the ahupuaʻa[1] of Kealakekua in the Kona moku[2] of Hawaiʻi mokupuni[3] on the slopes of Maunaloa[4], 1,450 m above sea level. Overall, the slope does not exceed 5 %, but slope is locally heterogenous due to the underlying lava flow terrain. This area is considered lower montane dry forest (Asner et al., 2005), with mean wet season (i.e., summer in Kona) high and low temperatures of 28.3 and 21.1 °C, respectively; dry season (i.e., winter in Kona) high and low temperatures of 24.6 and 10.1 °C, respectively; and mean annual precipitation is 736 mm (U.S. Climate Data, 2021). The experimental site is on a lava flow estimated to be 1,500 – 3,000 years old (Sherrod et al., 2021). Soils are a mixture of Puʻukala medial silt loams (medial-skeletal, amorphic, isomesic Lithic Haplustands), and Kēkake organic soils (euic, isomesic, micro Lithic Ustifolists). Both are shallow and moderately- to well-drained soils formed in variable amounts of organic material mixed with basic volcanic ash over pāhoehoe lava (NRCS, 2021).

2.3.2 Plant Material

The three native plants used in this experiment were ʻiliahi, koa, and ʻaʻaliʻi (Table 2.1). The source used for all three species were open-pollinated seeds from Hawaiʻi Island (Kona moku[2]) and germinated based on a June 2019 planting date (Table 2.2). All seedlings were grown in hard-walled plastic containers at the Maui Native Tree Nursery in Kula, Hawaiʻi. Seedlings were sorted for consistently prior to planting.

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[1] ahupuaʻa: land division usually extending from the uplands to the sea (Ulukau, 2022)

[2] moku: district (Ulukau, 2022)

[3] mokupuni: island (Ulukau, 2022)

[4] Maunaloa: one of five shield volanoes comprising Hawaiʻi Island

Table 2.1 Common name, scientific name, family, host-parasite relationship, nitrogen-fixing, and stature at maturity for the three species in the experiment: ʻiliahi, koa, and ʻaʻaliʻi.

Common name	Scientific name	Family	Host-Parasite Relationship	Nitrogen-fixing	Stature at maturity
ʻiliahi	*Santalum paniculatum*	Santalaceae	Hemiparasite	No	Large tree
koa	*Acacia koa*	Fabaceae	Host	Yes	Large tree
ʻaʻaliʻi	*Dodonaea viscosa*	Sapindaceae	Host	No	Shrub/ small tree

Table 2.2 Common name, outplanting age (months), nursery container, initial height (cm) (± SE), and initial diameter (mm) (± SE) for the three species in the experiment: ʻiliahi, koa, and ʻaʻaliʻi. D40 and SC10 nursery containers are cylindrical containers with volumes of 656 and 164 mL, respectively (Stuewe & Sons, Inc., Tangent, OR, USA).

Common name	Outplanting age (months)	Nursery container	Initial height (cm)	Initial diameter (mm)
ʻiliahi	12	D40	45 (0.7)	4.7 (0.05)
koa	3	SC10	38 (0.6)	3.1 (0.05)
ʻaʻaliʻi	8	SC10	28 (0.5)	2.6 (0.04)

2.3.3 Experimental Design and Treatments

This experiment was established as a completely randomized design and structured with 40 columns spaced 3 m apart (Appendix 2.1). Columns were oriented 60 °/240 ° along the contour to account for site topography. Each column had nine rows spaced 6 m apart resulting in 360 experimental units. One of nine treatments, with 40 replicates per treatment, were randomly assigned to each experimental unit: ʻiliahi with (1) no woody host (control), (2) koa at <0.2 m, (3) koa at 0.5 m, (4) koa at 1.0 m, (5) koa at 2.0 m, (6) ʻaʻaliʻi at <0.2 m, (7) ʻaʻaliʻi at 0.5 m, (8) ʻaʻaliʻi at 1.0 m, and (9) ʻaʻaliʻi at 2.0 m. The planting distances were implemented within set ranges. The closest distance, <0.2 m, was as close as possible. The next distance was set between 0.25 - 0.5 m, followed by 0.75 - 1.0 m, and lastly 1.75 - 2.0 m. If the treatment had an assigned host, then the host was planted within the column with its respective treatment distance on the western (ma kai) side of the ʻiliahi seedling. This planting arrangement resulted in a uniform distance of 3 m between columns and a variable distance between experimental units within a column depending on the distance the host was planted from ʻiliahi; however, regardless of the ʻiliahi-host distance, the minimum distance between experimental units within a column was 4 m. Each column had a koa seedling at the eastern (ma uka) end to account for potential border host effects. There was a 5-m buffer between the experimental site and other reforestation plantings.

Site preparation was completed in June 2019. Using an excavator, the grass was removed, and the ground scraped to expose soil from lava rock. As the grass grew back, competition and potential parasitism with the grass were assumed to be homogenous across each ʻiliahi planting. One year after planting, a grass-specific herbicide (24.5% active ingredient fluazifop-P-butyl, Fusilade® DX, reg. no. 100-1070, Syngenta Crop Production LLC, Greensboro, NC, USA) was aerially applied to the experimental site and surrounding area to reduce grass competition.

2.3.4 Measurements

At the time of planting (June 2019), height (cm) to the base of the apical meristem and ground line diameter (mm) were measured for all seedlings (ʻiliahi and hosts). Survival, height, and diameter were re-measured three years after planting for all plants. Survival of both ʻiliahi and hosts was recorded as a binary response (i.e., alive or dead). A subsample of seedlings was used to assess ʻiliahi foliar nutrient status (i.e., N, P, K, Ca, Mg, S). For each subsample, 3-5 leaves from the upper one-third portion of the plant were collected and dried at 60°C for 48 h. Dried samples were composited, pulverized, and analyzed at the University of Hawaiʻi at Hilo Analytical Lab.

2.3.5 Statistical Analyses

All data were analyzed with R software version 4.2.0 (R Core Team, 2022). Because we included a no host control treatment, the experimental design was not a full factorial between host species and planting distance and we could not analyze the data with a two-way ANOVA. Therefore, the independent variable was treatment with nine levels. ʻIliahi survival was analyzed with a logistic regression. ʻIliahi height, diameter, and foliar nutrients were analyzed with one-way ANOVA with treatment combination as the fixed factor. Koa and ʻaʻaliʻi height and diameter were analyzed separately with one-way ANOVA with planting distance as the fixed factor. Regressions were used to compare ʻiliahi growth and host growth for each treatment. Residuals from all response variables were tested to ensure normality and homogeneity of variance. ʻIliahi diameter was log-transformed to meet assumptions. If a significant treatment effect was detected ($p \leq 0.05$), the multcomp package (Hothorn et al., 2008) was used to run Tukey's HSD test for pairwise comparisons ($\alpha = 0.05$ level).

The hemiparasitic nature of ʻiliahi added noteworthy methods and statistical considerations. First, ʻiliahi could be parasitizing the pasture grass; however, because grass cover was relatively uniform across the site, if the grass was parasitized, we assumed this equally affected all treatments. Second, we removed ʻiliahi from the dataset for instances where ʻiliahi survived, but its host died, n = 19. Third, we removed ʻiliahi from the dataset for instances where koa naturally regenerated and likely affected treatments by introducing an additional host(s), n = 7.

2.4 Results

Initial height and diameter of planted ʻiliahi were similar across all treatments with an overall mean height of 45 ±0.7 cm ($F_{8,343}$=1.27, p=0.257) and an overall mean diameter of 4.7 ±0.05 mm ($F_{8,343}$=1.90, p=0.585; Table 2.2). After three years, ʻiliahi survival was only significantly greater when paired with koa at 1.0 m distance was significantly greater than ʻiliahi with no host and ʻiliahi paired with koa at 2.0 m (Figure 2.1; $X^2_{8,344} = 22.20$, $p = 0.005$; Fig. 1).

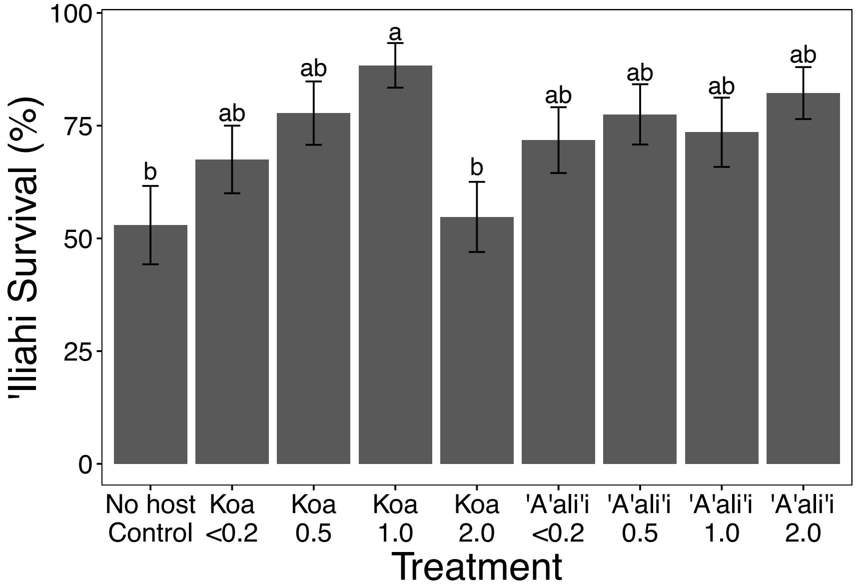

Figure 2.1 Mean percent survival (%) (± SE) of ʻiliahi seedlings three years after planting. ʻIliahi seedlings were planted either by itself with no host or with koa or ʻaʻaliʻi at four distances (<0.2, 0.5, 1.0, or 2.0 m away from ʻiliahi). Different letters indicate significant differences among the nine treatments ($\alpha = 0.05$).

There was a significant treatment effect for both height ($F_{8,246} = 9.77$, $p < 0.001$) and diameter ($F_{8,246} = 5.71$, $p < 0.001$). After three years, mean ‘iliahi seedling height and diameter were consistently greatest when paired with koa at <0.2 m (Figure 2.2). Height and diameter were next greatest with koa at 1.0 m. ‘Iliahi height and diameter when paired with ‘a‘ali‘i was comparable to the control (Figure 2.2).

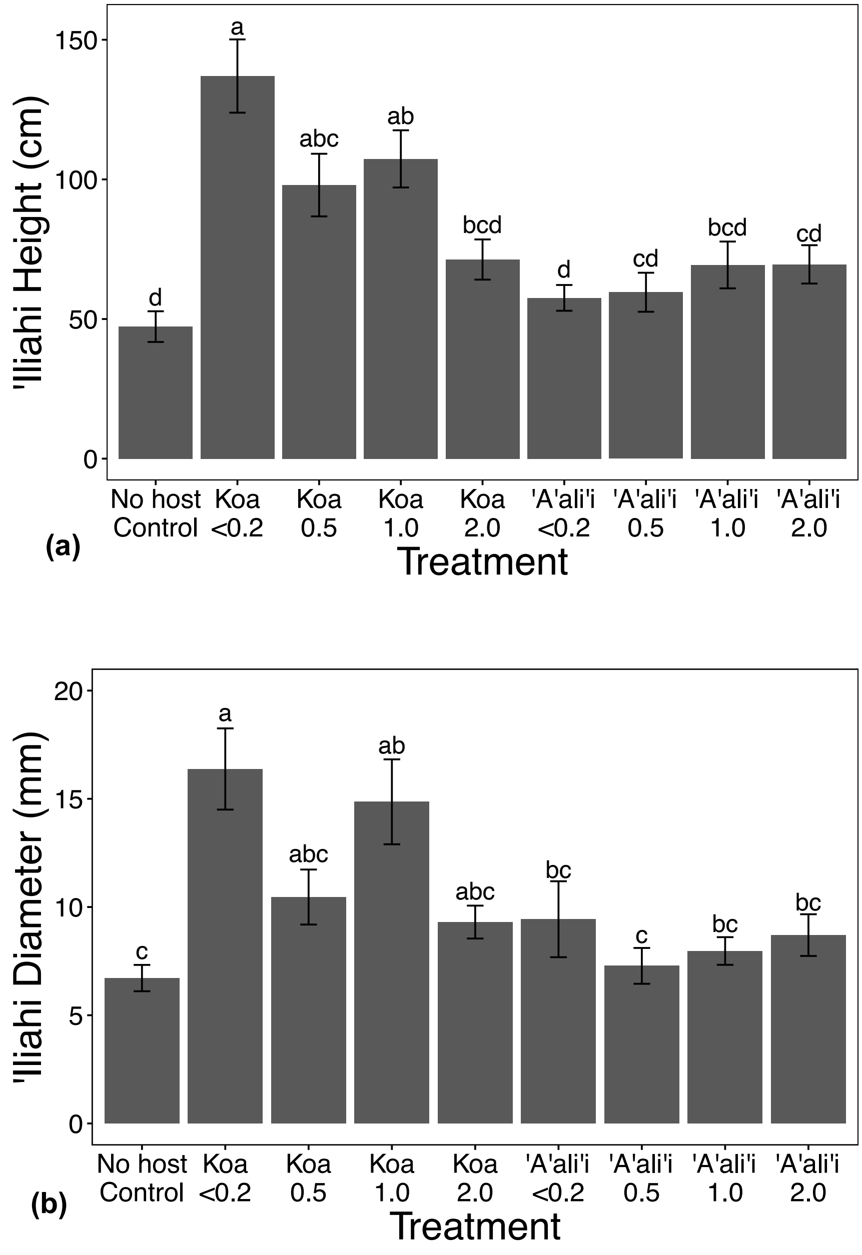

Figure 2.2 Mean **(a)** height (cm) and **(b)** diameter (mm) (± SE) of ‘iliahi seedlings three years after planting. ‘Iliahi seedlings were planted either by itself with no host, koa, or ‘a‘ali‘i at four distances (<0.2 m, 0.5 m, 1.0 m, or 2.0 m away from ‘iliahi). Different letters indicate significant differences among the nine treatments (α = 0.05).

For the host plants, mean host survival three years after planting was 85.6 % for koa and 91.9 % for ‘a‘ali‘i. Koa had greater mean (± SE) height and diameter (563.43 ±10.86 cm, 105.10 ±1.96 mm) than ‘a‘ali‘i (181.84 ±6.28 cm, 34.33 ±1.01 mm) three years after planting. The effect of host position did not affect height or diameter for either koa ($F_{3,101}$ = 1.48, *p* = 0.225; $F_{3,101}$ = 2.67, *p* = 0.058, respectively) or ‘a‘ali‘i ($F_{3,110}$ = 0.70, *p* = 0.553; $F_{3,110}$ = 1.40, *p* = 0.246, respectively), nor were there significant regression relationships between ‘iliahi growth and growth of either host when analyzed separately for each of the four distance treatments.

Mean concentration (%) of foliar nitrogen (N) was greater for ‘iliahi paired with koa at distances of <0.2, 0.5, and 1.0 m ($F_{8,142}$ = 4.68, *p* <0.001; Table 2.3). The other nutrients had different patterns with observed phosphorous (P) and calcium (Ca) greater for ‘iliahi paired with ‘a‘ali‘i than when paired with koa at certain distances ($F_{8,142}$ = 7.18, *p* <0.001; $F_{8,142}$ = 3.01, *p* = 0.004, respectively), potassium (K) greater for ‘iliahi without a host than with either host ($F_{8,142}$ = 4.78, *p* <0.001), and magnesium (Mg) and sulfur (S) greater for ‘iliahi without a host or with most ‘a‘ali‘i distances than with koa ($F_{8,142}$ = 5.09, *p* <0.001, $F_{8,142}$ = 2.62, *p* = 0.011, respectively; Table 3).

Table 2.3 Mean nutrient concentration (%) (± SE) for ‘iliahi paired either with no host, koa, or ‘a‘ali‘i three years after planting at <0.2, 0.5, 1.0, or 2.0 m distances. Nutrients include: nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S). Within each nutrient parameter, different letters indicate significant differences among the nine treatments (α = 0.05).

Host	Distance	N	P	K	Ca	Mg	S
Control No host	—	1.20 (0.12) b	0.13 (0.02) abc	3.72 (0.36) a	0.62 (0.04) ab	0.38 (0.02) a	0.29 (0.03) a
Koa	<0.2	2.31 (0.14) a	0.10 (0.01) c	1.62 (0.18) b	0.30 (0.06) b	0.14 (0.03) c	0.12 (0.01) b
Koa	0.5	2.33 (0.19) a	0.10 (0.01) c	2.29 (0.29) b	0.46 (0.07) ab	0.21 (0.03) bc	0.18 (0.02) b
Koa	1.0	2.56 (0.33) a	0.12 (0.01) bc	2.21 (0.21) b	0.48 (0.08) ab	0.21 (0.03) bc	0.18 (0.01) b
Koa	2.0	1.90 (0.52) ab	0.10 (0.04) bc	2.59 (0.22) b	0.45 (0.04) ab	0.27 (0.03) bc	0.17 (0.01) b
‘A‘a‘li‘i	<0.2	1.44 (0.20) b	0.17 (0.04) a	3.12 (0.25) b	0.69 (0.09) a	0.37 (0.05) abc	0.25 (0.07) ab
‘A‘a‘li‘i	0.5	1.74 (0.25) ab	0.14 (0.04) ab	3.05 (0.36) b	0.61 (0.06) ab	0.29 (0.04) abc	0.19 (0.02) b

‘A‘a‘li‘i	1.0	1.99 (0.22) ab	0.21 (0.04) a	2.64 (0.44) b	0.51 (0.10) ab	0.37 (0.07) abc	0.26 (0.05) ab
‘A‘a‘li‘i	2.0	1.65 (0.22) ab	0.16 (0.04) ab	3.13 (0.46) b	0.68 (0.10) a	0.40 (0.05) ab	0.24 (0.04) ab

Throughout this experiment, we observed great variability between how ‘iliahi responded within a treatment (Appendix 2.2). Each treatment had at least one ‘iliahi seedling that experienced proficient growth and at least one ‘iliahi with slow growth rates. Overall, koa pairings resulted in higher ‘iliahi survival and growth compared to ‘a‘ali‘i and the no host control; furthermore, shorter distances to the koa host also generally resulted in greater growth.

2.5 Discussion

For restoration sites with no pre-established hosts, koa co-planted at a shorter distance was a key advantage for early ‘iliahi growth, whereas koa co-planted at an intermediate distance was advantageous for ‘iliahi survival. Greater survival and growth results for ‘iliahi paired with koa partially support hypothesis (i) that ‘iliahi performance would be greater with a host than with no host. In full support of hypothesis (ii), ‘iliahi seedling growth and foliar nitrogen were greater when paired with koa (the nitrogen-fixing host) than with an ‘a‘ali‘i host (Figure 2.2; Table 2.3). For ‘iliahi paired with ‘a‘ali‘i, there was no significant difference for survival, height, or diameter (Figure 2.1; Figure 2.2). This lack of difference may indicate a reduced host suitability or at least comparable to the effect of the pasture grass. ‘A‘ali‘i potentially possesses characteristics (e.g., allelochemicals) that may restrain the growth of other plants (Rowshan et al., 2014). Additionally, ‘a‘ali‘i shrub characteristics may affect ‘iliahi differently than koa, such as foliar shade affecting light quality. In addition to being reported as a suitable host for Indian sandalwood, ‘a‘ali‘i has been ambiguously reported as an unsuitable host to Indian sandalwood (Rocha and Santhoshkumar, 2022) and African sandalwood (Mwang’ingo et al., 2005).

The ability of koa to promote greater ‘iliahi performance as a host is likely due to koa being a nitrogen-fixing tree. ‘Iliahi’s greater foliar nitrogen concentration when paired with koa (Table 2.3) is likely advantageous and contributed to increased height and diameter growth for ‘iliahi paired with koa (Figure 2.2). The benefit of a nitrogen-fixing host has been identified for other hemiparasitic plants (Irving and Cameron, 2009; Matthies, 2017; Nge et al., 2019) including other *Santalum* spp. (Brand et al., 2000; Lu et al., 2014; Nge et al., 2019; Radomiljac et al., 1999; Veillon and Jaffré, 1995; Woodall and Robinson, 2003). Since nitrogen is noted as an important resource hemiparasites acquire from hosts (Cameron and Seel, 2007; Lu et al., 2014; Pageau et al., 2003; Tennakoon et al., 1997a), nitrogen transfer is a likely benefit from nitrogen-fixing trees. Koa's host suitability could also be due to other characteristics, such as fast growth and development (Scowcroft et al., 2007) leading to a higher probability of haustoria connections. Rapid growth of the host (e.g., koa) during the establishment phase also seems to be of a net benefit that outweighs any competitive inhibition to hemiparasites (Hautier et al., 2010; Radomiljac et al., 1998). Additionally, koa's fast growth and canopy development may facilitate ʻiliahi establishment by ameliorating harsh site conditions (e.g., high light, dry periods), thereby increasing ʻiliahi performance.

Given the hemiparasitic nature of ʻiliahi*,* there are likely additional ecological trade-offs between competition with the host for resources and facilitation through root parasitism (Keith et al., 2004; Matthies, 1995). In partial support of our final hypothesis (iii), ʻiliahi growth when paired with koa was greatest at the closest distance (Figure 2.2). This result aligns with host considerations for Indian sandalwood of maximizing functional haustoria and minimizing competition for aboveground resources (Rocha et al., 2014). Earlier connections forged between the closest pairings could explain the koa host distance effect. For Indian sandalwood, earlier connections forged between a nursery container host yielded greater growth after planting (Radomiljac et al., 1998). The intermediate spacings, however, may be close enough to encourage early connections and sufficient growth enhancement, yet not too close where seedlings quickly become intertwined and compete for limited resources, such as light. The intermediate spacings also align to the growers' guide for Vanuatu sandalwood (Page et al., 2012). The response from 0.5 m spacing may indicate reduced haustoria connections (compared to <0.2 m) and increased competition (compared to the 1.0 m). The 1.0 m intermediate spacing with koa was also the only significantly different treatment for ʻiliahi survival results compared to no host control or koa at 2.0 m (Figure 2.1). The difference between ʻiliahi survival for 1.0 and 2.0 m koa distances may be indicative of the greater distance lacking haustoria benefits. This result also supports that while hemiparasites can survive independently of a woody host, suitable long-term hosts may increase survival and growth of the hemiparasite (Těšitel, 2016). The different response of survival and growth to distance may similarly be due to trade-off differences between benefits from haustoria formation and competition from hosts.

While parasitic plants have the potential to affect the plant communities that they inhabit (Cameron and Phoenix, 2013), similar to Brand et al. (2000), we did not detect an influence of ‘iliahi on the survival or growth of the hosts themselves. It is interesting that even though there was an effect of distance for ‘iliahi paired with koa (Figure 2.2), there was not an apparent negative effect of parasitism on koa growth. Perhaps the effect of ‘iliahi on host plant survival and growth is beyond the scope of early establishment. Nitrogen-fixation on site by koa is highly likely because nitrogen was the prominent exception to the trend of ‘iliahi with no host having greater foliar nutrient concentration than ‘iliahi paired with either host (Table 2.3). Lower observed P, K, Mg, and S concentrations in ‘iliahi paired with a host could potentially be due to a dilution effect of ‘iliahi growing faster with hosts. This result could also be due to the complexity behind ‘iliahi acquiring nutrients auto- and heterotrophically as seen with Indian sandalwood and two nitrogen-fixing hosts in a plantation setting (Lu et al., 2020). Especially given studies that support transfer of several other mineral nutrients (e.g., Ca, K, P, Na, Cu) between *Santalum* spp. and hosts when a hemiparasitic connection is made (Radomiljac et al., 1999b; Struthers et al., 1986). Management of mixed species stands with ‘iliahi need to consider several future directions depending on site goals. For example, deciding whether to keep, remove, or prune hosts if hosts are too competitive for resources (e.g., light, mineral nutrients) or entangled with ‘iliahi. Further work on this experimental site must account for root systems expanding past the three m spaced rows and six m spaced planting positions; therefore, considering a larger experimental scale of interacting trees and an increasing role of competition both above- and below-ground would be beneficial to following the trees over time.

2.6 Conclusions

Our research contributes to knowledge of hemiparasitic plants broadly regarding the role of hosts and co-planting proximity of hemiparasites to their host. Few studies have experimentally examined host distance, and our results support those of Keith et al. (2004) that the effect of distance depends on host species. In our study, koa was a more effective host for early ‘iliahi establishment than ‘a‘ali‘i. Host proximity has costs and benefits to hemiparasites with respect to trade-offs between competition from and proximity to a suitable host. Even though the closest distance for ‘iliahi paired with koa resulted in the greatest growth, planting at an intermediate distance (e.g., 1.0 m) still yielded increased growth relative to the farthest distance (2.0 m) and provides the plants physical room to grow and avoid entanglement. Consideration of secondary hosts at farther distances will likely be necessary for continued survival and growth of ‘iliahi beyond the early establishment phase. Additional research may help to understand how unaccounted for site effects (e.g., soil characteristics, microclimate, soil compaction) may interact with host species and co-planting distances to increase variation within the results.

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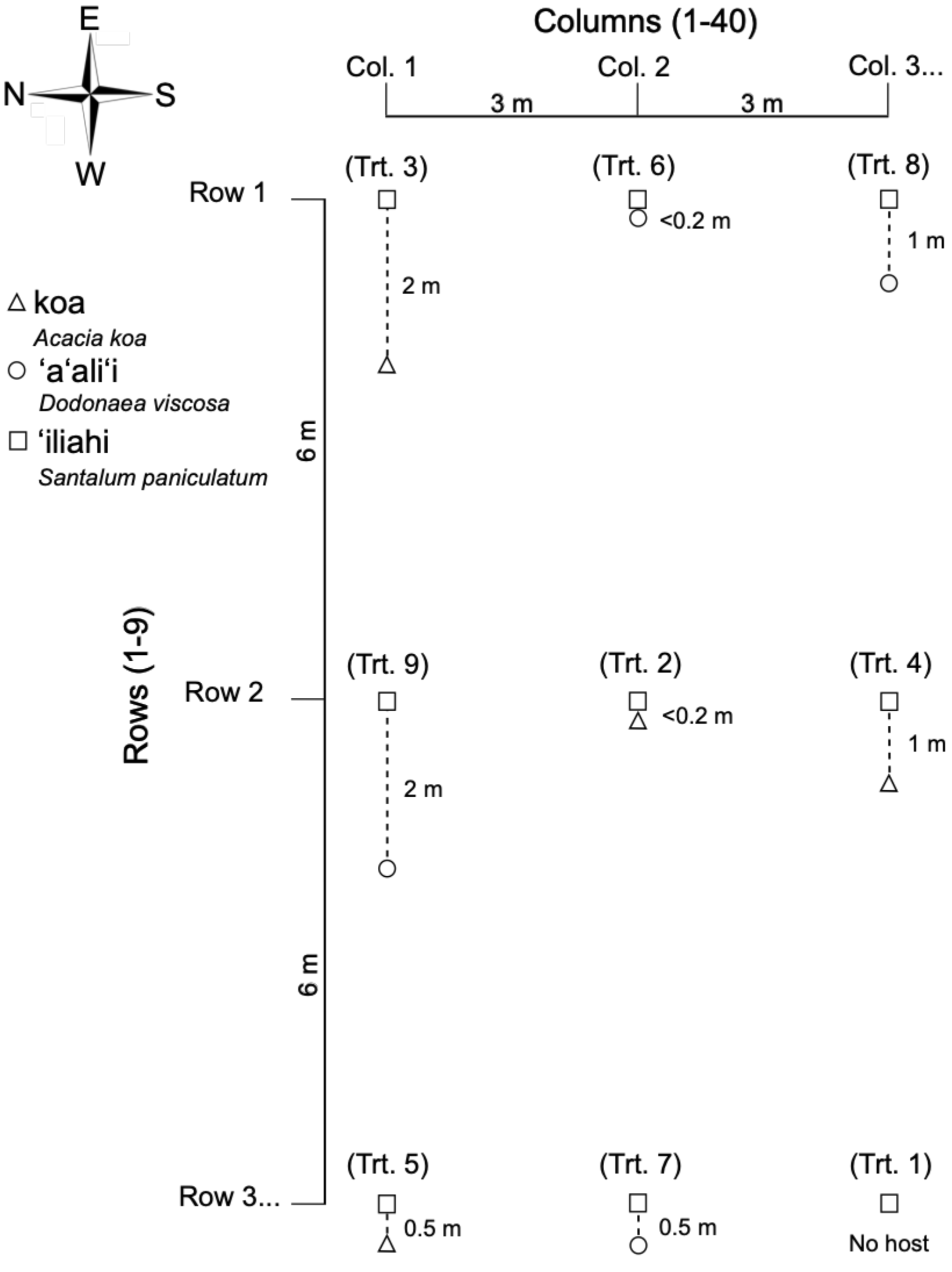
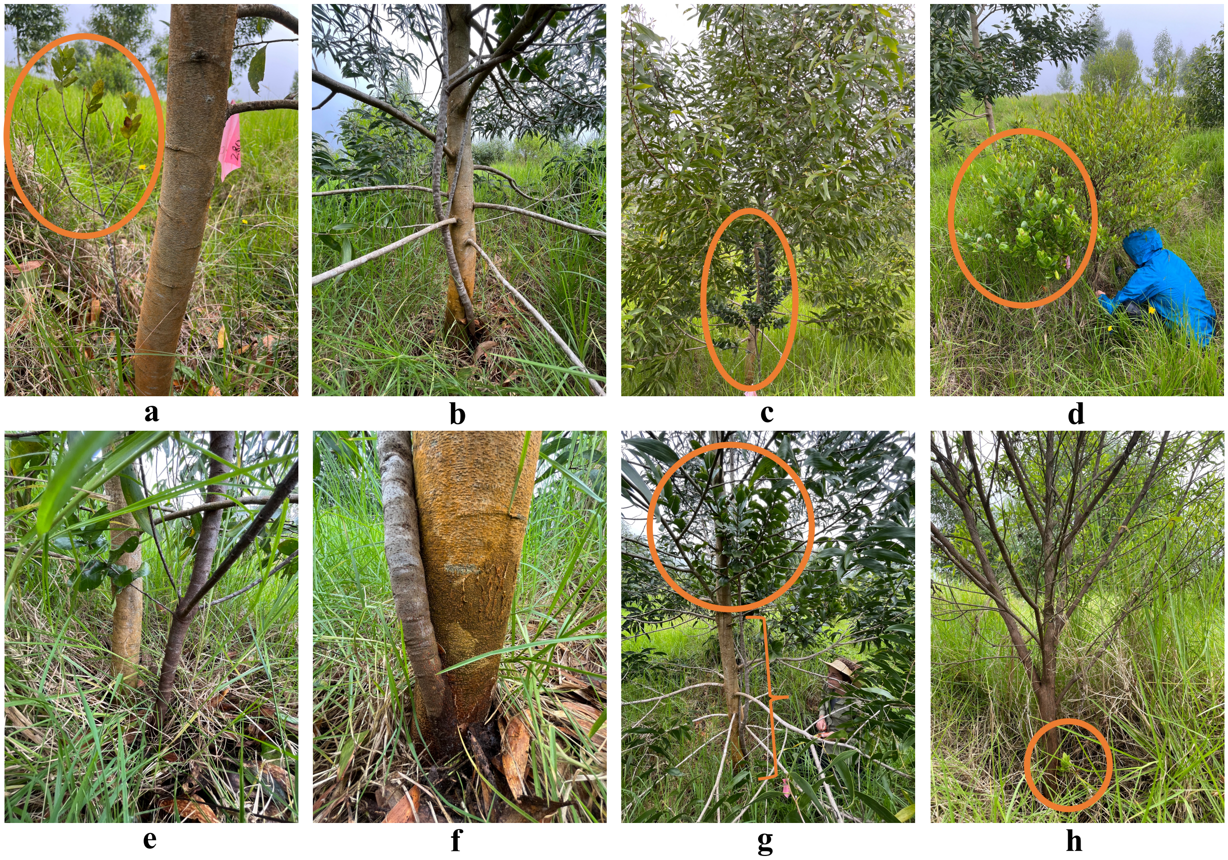
2.9 Appendix 2

Appendix 2.1 Example first three rows of the first three columns to illustrate the experimental design and treatment layout. There was uniformly 3 m between columns and 6 m between ‘iliahi seedlings within a column. There was minimally 4 m between ‘iliahi and the next experimental units host within the same column. Distance between ‘iliahi seedlings and the host of the next experimental units within a column depended on the host distance for the treatment randomly assigned one of the 360 experimental units. Treatments (Trt.) included ‘iliahi with (1) no woody host (control), (2) koa at <0.2 m, (3) koa at 0.5 m, (4) koa at 1.0 m, (5) koa at 2.0 m, (6) ‘a‘ali‘i at <0.2 m, (7) ‘a‘ali‘i at 0.5 m, (8) ‘a‘ali‘i at 1.0 m, and (9) ‘a‘ali‘i at 2.0 m. If the treatment had an assigned host, then the host was planted within the column with its respective treatment distance on the western side of the ‘iliahi seedling.



Appendix 2.2 ‘Iliahi 3 years after planting paired either with koa or ‘a‘ali‘i as hosts. ‘Iliahi has a grey coloration compared to an orange color for koa and a brown color for ‘a‘ali‘i. Orange circles added to distinguish ‘iliahi. **a)** ‘Iliahi performing poorly when paired with koa 0.5 m apart **b)** ‘Iliahi performing very well when paired with koa <0.2 m apart **c)** ‘Iliahi performing very well when paired with koa 0.5 m apart **d)** ‘Iliahi performing very well when paired with ‘a‘ali‘i <0.2 m apart **e)** ‘Iliahi performing well when paired with koa 0.5 m apart **f)** close up of b), concern of long-term effects **g)** ‘Iliahi performing very well when paired with koa <0.2 m apart **h)** ‘Iliahi performing poorly when paired with ‘a‘ali‘i <0.2 m apart (Photos: Emily Thyroff and Quinn Moon, 2022).



Chapter 3 – Underplanting a Hemiparasitic Tropical Tree Beneath an Established Host Species Canopy Mitigates Tradeoffs Between Access to Root Networks and Light

3.1 Abstract

Silvicultural techniques of underplanting or using nurse trees can be beneficial for the regeneration of certain species, particularly later successional, more shade-tolerant species, and species dependent upon other plants. At a site previously reforested with koa (*Acacia koa*), we evaluated whether ʻiliahi could be underplanted to diversify the initial planting and to examine how ʻiliahi performs given its hemiparasitic acquisition of resources from hosts. We hypothesized that there is an optimal range of overstory gap sizes that balances competition for resources and having abundant root networks for ʻiliahi to establish hemiparasitic root connections. Two years after planting, overall ʻiliahi seedling survival was very high (97.5 %) and not affected by overstory canopy openness. ʻIliahi seedling height and diameter, however, were greater with larger canopy openness. Photosynthetic parameters (light compensation and light saturation) and stomatal density also increased with greater canopy openness. Our positive establishment results contribute to our understanding of ʻiliahi regeneration as a hemiparasitic tree. Our results also help improve silvicultural efforts by demonstrating successful ʻiliahi underplanting in a range of canopy openness. Having an established host yet sufficient canopy openness can help restore functionally compatible and abundant ʻiliahi regeneration into forests.

3.2 Introduction

Underplanting seedlings in established forests, using nurse trees, and enrichment plantings are silvicultural techniques that can help to restore tree and other forest species where natural regeneration is limited (Paquette et al. 2006; Dey et al. 2012; Maltoni et al. 2019; Thyroff et al. 2019; Rose et al. 2019). Underplanting may facilitate seedlings in overcoming limiting factors for species that are later successional, shade-tolerant, or susceptible to browse (Dey et al. 2012; Maltoni et al. 2019; Thyroff et al. 2019; Rose et al. 2019). Single-species reforestation or natural regeneration with only one or a few dominant species can be diversified by underplanting (Paquette et al. 2006; Messier et al. 2022). Underplanting may also be particularly beneficial for species with unique relationships with other plants, such as parasitic or hemiparasitic plants because of their dependence on host root networks.

Approximately 1% of all angiosperms are parasitic, and most are root hemiparasites (Heide-Jørgensen 2013; Matthies 2017). Hemiparasitic plants are capable of photosynthesis yet rely on their hosts to acquire resources such as water and nutrients for long-term survival (Bell and Adams 2011; Matthies 2017). Planting hemiparasites under established host plants, rather than planting hemiparasites and hosts simultaneously, may allow the hemiparasites to connect more quickly to host root systems and increase the capacity of resource supply from hosts. There may be tradeoffs, however, between the benefits of resource transfer from the host root system and competition with the host, especially for light (Matthies 1995). Thus, there is likely to be a host plant density to balance resource supply with overstory shading, depending at least partially on the ecophysiology of the underplanted species (Soto et al. 2017; Rose et al. 2019). Furthermore, shade tolerance of underplanted hemiparasitic trees may be dynamic; for example, a *Santalum album* L. (Indian sandalwood) study reported a range of light environments as seedlings progress through early development (Barrett and Fox 1994). Seedlings in the highest shade treatment (80 % shade) had greater survival and adaptive characteristics to sustain in shaded environments such as leaf area, leaf thickness, petioles, and chlorophyll (Barrett and Fox 1994).

Hemiparasitism in Hawaiian forests has been minimally studied, yet hemiparasitism was likely a major forest relationship based upon large estimated historical ranges of endemic *Santalum* spp. (Price et al. 2012), locally known as ʻiliahi or Hawaiian sandalwood. *Santalum* spp. occur throughout Oceania, with six of the 20 species endemic to Hawaiʻi (Wagner et al. 1999; Harbaugh et al. 2010; Teixeira da Silva et al. 2016). Internationally, including in Hawaiʻi, *Santalum* spp. have been overharvested as valued sources of sandalwood oil and wood (Thomson et al. 2011). ʻIliahi was historically abundant in dry forests, but this forest type in Hawaiʻi has been reduced by 90 % with only dispersed parcels remaining (Medeiros et al. 2014). Natural regeneration of ʻiliahi is uncommon due to depleted seed banks, limited remaining forest parcels, lack of seed dispersers, seed predators, and non-native herbivores. Given this deforestation legacy in Hawaiʻi and the unique hemiparasitic relationship, reforestation of ʻiliahi at degraded tropical dry forest sites also requires supporting host plant communities. Within the broader underplanting framework, ʻiliahi are anecdotally described as having limited shade tolerance (Merlin et al. 2006). In general, increased light often encourages the establishment of species in wet forests of Hawaiʻi, but in dry forests, full sun may be detrimental due to concomitant increases in temperature and evapotranspirational demand (Cordell et al., 2002; Inman-Narahri et al., 2013).

Due to its history and value, including cultural and ecological services and functions, there is significant local interest in restoring native Hawaiian tropical dry forests, including ‘iliahi regeneration. One native plant that ‘iliahi species often pair well with is koa (*Acacia koa* A. Gray) (Merlin et al. 2006; Thomson et al. 2011; Chapter 2). *Acacia* spp., overall, are identified as suitable nitrogen-fixing hosts for other sandalwood species (Tennakoon et al. 1997; Radomiljac et al. 1999; Brand et al. 2003; Woodall and Robinson 2003; Nge et al. 2019). Koa’s nitrogen fixation, fast growth, high water-use-efficiency, presence across large rainfall gradients, and tolerance to many abiotic factors (Gugger et al. 2018; Rose et al. 2019) increase its potential as a host for ‘iliahi. Koa is highly valued culturally and economically, and it is planted widely to increase native bird habitat in forests previously converted to pastures (Friday et al. 2006; Pejchar and Press 2006; Gugger et al. 2018). Due to its rapid early growth, ability to overtop weeds, and relatively low shading cast by its canopy, koa is often planted at reforestation and restoration sites to establish a relatively open canopy for future underplanting of native species (Pearson and Vitousek 2001; Scowcroft et al. 2008; Funk and McDaniel 2010; Scowcroft and Yeh 2013).

While hemiparasitism has been researched for other sandalwood species, literature on ‘iliahi is sparse. We aimed to improve the survival and establishment of planted ‘iliahi (*Santalum paniculatum* Hook. & Arn., endemic to Hawai‘i Island) seedlings by better understanding how hemiparasitic trees perform when planted under established host canopies. We predicted that certain host canopy openness and spacing would result in better ‘iliahi performance associated with a suitable balance between competition and parasitism for ‘iliahi seedlings and established koa hosts. With a denser overstory canopy and closer distance to the nearest host, competition between ‘iliahi and koa for light, water, and nutrients may offset the increased opportunities for resource transfer via root parasitism. By contrast, lighter overstory canopy and farther distance to the nearest host may result in a less dense root network that reduces opportunities for root parasitism, and could result in excessive light and ground temperatures leading to high evapotranspiration rates for young seedlings during the dry season. Thus, we predicted ‘iliahi underplanted among established koa with an intermediate canopy openness will have better survival and growth due to access to koa roots and minimal competition for light, water, and nutrients. We also predicted that gas exchange parameters and stomatal density would align with growth responses.

3.3 Materials and Methods

3.3.1 Experimental Site

This experiment was conducted at Kealakekua Mountain Reserve, an approximately 3,900-ha property designated for tropical dry forest restoration and sustainable forestry where forest harvesting, pasture establishment, and grazing have historically inhibited natural regeneration of native forest species, including ʻiliahi and koa. Kealakekua Mountain Reserve is within the ahupuaʻa[1] of Kealakekua in the Kona moku[2] of Hawaiʻi mokupuni[3] on the slopes of Maunaloa[4], 1,450 m above sea level. This area is considered lower montane dry forest (Asner et al., 2005), with mean wet season (i.e., summer in Kona) high and low temperatures of 28.3 and 21.1 °C, respectively; dry season (i.e., winter in Kona) high and low temperatures of 24.6 and 10.1 °C, respectively; and mean annual precipitation of 736 mm (U.S. Climate Data, 2021). Soils at this site are Puukala medial silt loams, amorphic, isomesic Lithic Haplustands, which are shallow and well-drained soils that formed in basic volcanic ash over pāhoehoe lava (NRCS, 2021).

In July 2010, a 0.81-ha plot was fenced within an actively grazed area to plant koa seedlings for native bird habitat (N 19.4981°, W -155.8082°). Pasture grass (*Cenchrus clandestinus* Hochst. ex Chiov.) was uniformly present in the fenced area. Two hundred and seventy-one koa seedlings grown from local seed sources were planted in the enclosure along planting rows previously sprayed with herbicide to control the pasture grass. Survival was only 77 % after the first year, likely due to 2010 being one of the driest years on record for Hawaiʻi Island. Ten years after the koa planting (August 2020), survival was 59 %. Koa seedling mortality resulted in varying canopy gap sizes, creating a matrix of canopy openness and resulting understory micro-environments. Of the 160 surviving koa, height and diameter at breast height (DBH, taken at 1.37 m) were measured for 68 individual koa (42.5 % of all koa trees in the plot).

3.3.2 Plant Material and Experimental Design

Nine-month-old ʻiliahi seedlings grown in 760 cm^3 rectangular containers (028PIFD, Proptek, Belleville, MI, USA) were planted into the field site in August 2020. Seedlings were obtained from Hāloa ʻĀina Reforestation Project nursery in Kealakekua, Hawaiʻi and grown from a local seed source. From baseline morphology analysis (n = 20), mean (± SE) seedling diameter was

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[1] ahupuaʻa: land division usually extending from the uplands to the sea (Ulukau, 2022)

[2] moku: district (Ulukau, 2022)

[3] mokupuni: island (Ulukau, 2022)

[4] Maunaloa: one of five shield volanoes comprising Hawaiʻi Island

5.5 ±0.2 mm, height was 31 ±1 cm, and root to shoot dry mass (g) ratio was 0.49 ±0.02. Seedlings were sorted for consistency prior to planting and then randomly assigned a planting site. Seedlings were hand planted with planting bars.

We planted ‘iliahi beneath the previously planted koa with the resulting gradient of canopy openness for underplanted ‘iliahi. ‘Iliahi seedlings were planted on a 78 × 78-m grid with 4.5 m between planting sites. The grid consisted of 18 planting sites within 18 transects yielding a total of 324 independent experimental units. To maintain planting density and intra-specific seedling competition, a perimeter of buffer ‘iliahi seedlings was planted 4.5 m from the research seedlings around the entire plot. Pasture grass was mechanically cut back (i.e., using brush saws and hand clippers) before planting and every three months for the first year of growth.

3.3.3 Measurements

At the time of planting (August 2020), ‘iliahi height to the base of the apical meristem (cm) and ground line diameter (mm) were recorded. Two years after planting (August 2022), survival and growth (height and diameter) were recorded. ‘Iliahi survival was recorded as a binary response; “alive” included seedlings with any green leaves.

At every ‘iliahi seedling, hemispherical photos were taken with a Sony Mirrorless Digital Camera NEX Series camera (Sony Corporation, Tokyo, Japan) using a Madoka 180 hemispherical lens (Yasuhara, Hiroshima, Japan). All photos were taken 1 m above, at a zero-degree zenith, oriented north, using a tripod-mounted level under homogeneous diffuse sky conditions to quantify the canopy environment. Photographs were analyzed with CIMES software (Gonsamo et al. 2011) to determine percent canopy openness. Based upon the determined canopy openness, seedlings were assigned to five categories (0-20 %, 20-40 %, 40-60 %, 60-80 %, 80-100 %). From these categories, a subsample of 16 seedlings were randomly selected for physiological measurements to ensure a representation of seedlings along the continuous canopy openness, resulting in 80 total sampling units. Additionally at each seedling planting site, the distance from ‘iliahi to the nearest koa was recorded.

Leaf gas exchange and stomatal density were measured one year after planting (August 2021). Leaf gas exchange was measured with a portable LI-6800 (LI-COR Biosciences, Lincoln, Nebraska) to create light response curves. One upper-canopy, fully expanded, recently mature leaf per tree was measured between the hours 10:00 and 14:00. Light response curves were determined by measuring net CO_2 assimilation from 0-1,600 μmol m^{-2} s^{-1}. Infrared gas analyzers of the LI-6800 (IRGAs; reference and sample) were matched at the beginning and end of each light curve measurement. Relative humidity (~ 60%), vapor pressure deficit (<3.0 kPa), and temperature (leaf and block) were monitored for consistency. The gas exchange data point was taken after sample gas values (H_2O and CO_2) and net CO_2 assimilation were stable, based on coefficient of variation. If ʻiliahi leaves did not completely fill the 3 × 3 cm LI-6800 leaf chamber, gas exchange measurements were adjusted for actual leaf areas. Leaf areas were determined from a photo of the leaf in the chamber using ImageJ (National Institutes of Health, Bethesda, Maryland). Light response curves were created by plotting net CO_2 assimilation (A_N, μmol CO_2 m^{-2} s^{-1}) against PAR. The curves were fitted to a non-rectangular hyperbola (SigmaPlot V11.0, Systat Software, San Jose, California). Methodology to calculate final parameters from the model followed Chartier and Prioul (1976). Final parameters were used to calculate light compensation and light saturation points (μmol m^{-2} s^{-1}).

Stomatal density was calculated for the same 80 ʻiliahi seedlings used for gas exchange measurements using the same upper canopy, fully expanded, recently mature leaf. Impressions of the adaxial and abaxial leaf surfaces were made in the middle of each leaf, midway between the midrib and the leaf margin. Leaf impressions were made on microscope slides using cyanoacrylate. Five leaf impression images (DCM 900 microscope CMOS Camera, Oplenic Optronics, Hangzhou, China) were taken of a 0.19 × 0.14 mm (0.0266 mm^2) area under 40x magnification using a microscope (BH-2 microscope, Olympus, Tokyo, Japan). Stomatal counts were conducted using ImageJ and the cell counter plug-in (Kurt De Vos, University of Sheffield). For unbiased counting, all whole stomata were counted within the impression image area and stomata partially within the image were only counted on the top and right sides of the image area. Stomatal density (mm^{-2}) was calculated by dividing the number of stomata in the image by the image area.

3.3.4 Statistical analyses

All data were analyzed with R software version 4.2.0 (R Core Team 2022). Polynomial regression models were used for growth and physiological measurements with total canopy openness as the continuous independent factor and distance to nearest koa as a covariate. Fitted curves for each response variable were used to estimate the nature and significance of the relationship at $\alpha = 0.05$ level. Residuals from all response variables were tested to ensure normality and homogeneity of variance.

3.4 Results

3.4.1 Plot Characteristics

Ten years after the koa seedlings were planted, mean (± SE) koa DBH was 17.9 ±0.59 cm and mean height was 10.1 ±0.20 m. Canopy openness derived from the hemispherical photographs had a mean value of 35.9 % and a range of 8.8 % to 90.1 %. Canopy openness varied throughout the underplanting grid (Figure 3.1); however, there were more instances of lower canopy openness indicated by skewness of the distribution of canopy openness (Appendix 3.1). The mean distance of ‘iliahi planting sites to the nearest koa was 3.29 m with a range of 0.4 m and 13.0 m.

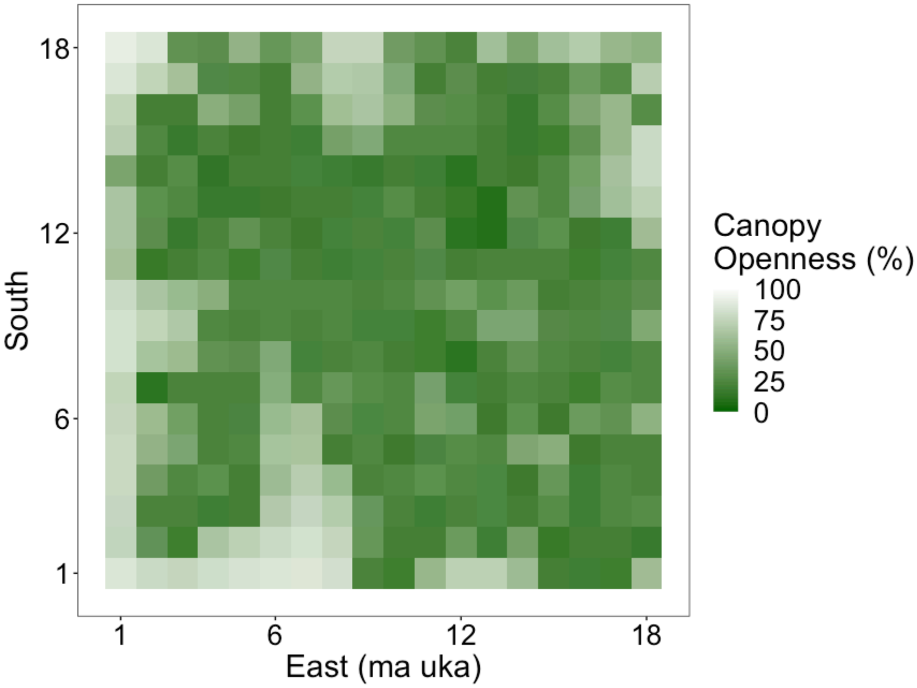


Figure 3.1 Heat map of the field site. Each square represents an ‘iliahi seedling. Canopy openness ranged from 8.8 % to 90.1 % with darker squares representing lower canopy openness and lighter squares representing higher canopy openness.

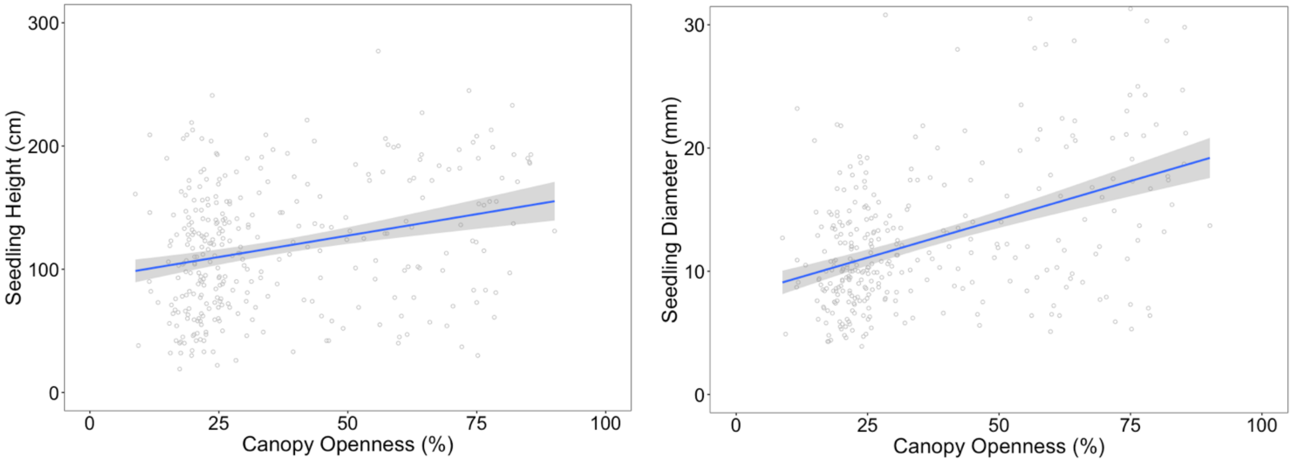
3.4.2 Seedling Performance

Initial height and diameter of planted ‘iliahi were similar across canopy openness (Table 3.1). After two years, ‘iliahi survival was 97.5 %; that is, of the 324 planted, only 8 died. After two years, height ranged from 19 to 277 cm and ‘iliahi diameter ranged from 3.9 to 36.6 mm. Both height and diameter had significant positive linear relationships with canopy openness. Significance was only at the first-order linear term and relatively low R^2 values (Figure 3.2; Table

3.1). For the height model, the distance to nearest koa covariate did not have a significant effect ($F_{1, 322} = 2.16$, $p = 0.142$) and did not add additional information to the model, therefore, the covariate was dropped. For the diameter model, the covariate did have a significant effect ($F_{1, 322} = 11.90$, $p < 0.001$) and added information to the model; therefore, the covariate was maintained.

Table 3.1 Linear model equations, F value, degrees of freedom (DF), P value, and R^2 derived for height and diameter at the time of planting and two years after planting. The "canopy" term in the linear model equation stands for canopy openness. The "distance" term in the linear model equation stands for distance to nearest koa.

Time	Linear model	F value	DF	P value	R^2
Initial	Height = -2.22*canopy+29.80	2.65	1, 322	0.104	0.005
	Diameter = -0.51*canopy +4.57	4.23	1, 322	0.141	0.010
2 years	Height = 68.56*canopy+93.18	25.01	1, 314	<0.001	0.071
	Diameter = 12.42*canopy+distance+8.00	76.39	1, 314	<0.001	0.193

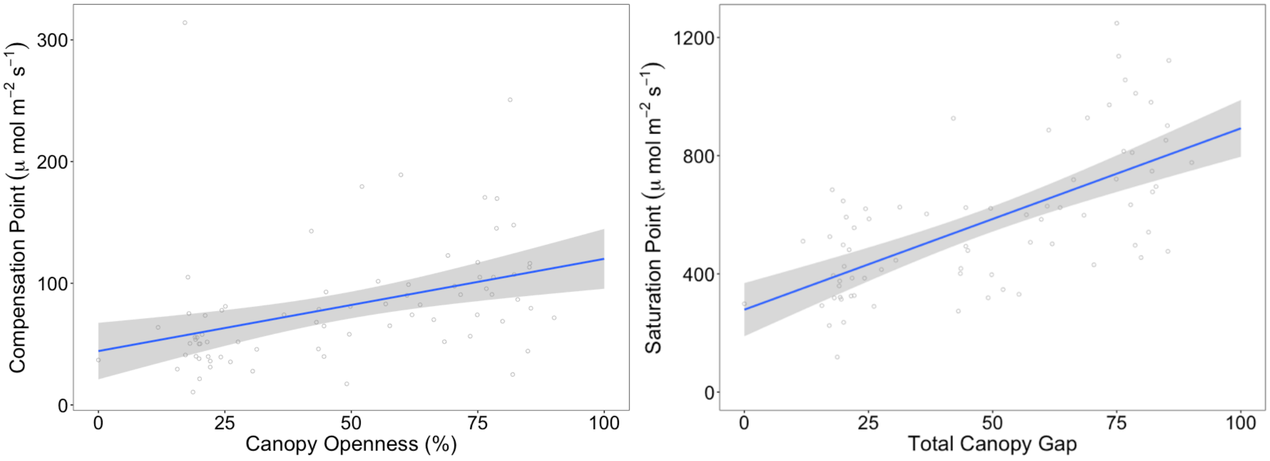


(a) **(b)**

Figure 3.2 **(a)** Height (cm) and **(b)** diameter (mm) growth by canopy openness (8.8 % to 90.1 %) of ʻiliahi seedlings 2 years after planting.

3.4.3 Physiology Measurements

For gas exchange parameters, there were significant positive linear relationships for both light compensation ($F_{1, 73} = 11.64$, $p = 0.001$) and light saturation points ($F_{1, 73} = 51.69$, $p < 0.001$) with canopy openness, but also only at the first-order linear term (Figure 3.3). Though both were significant, the relationship was relatively strong for light saturation point ($R^2 = 0.41$) and lower for light compensation point (R^2 = 0.13). Light response curves followed a similar trend (Figure 3.4). From the leaf impressions, there were detected stomata on the abaxial (i.e., lower) leaf surface and stomatal density ranged from 7 to 33 mm^2. There were no detected stomata on the adaxial (i.e., upper) leaf surface. There was a significant positive relationship between stomatal density and canopy openness (Figure 3.5; $F_{1,73}$ = 11.64, p = 0.001, R^2 = 0.40).



(a) **(b)**

Figure 3.3 Light compensation (μmol CO_2 m^{-1} s^{-1}) and light saturation point (μmol CO_2 m^{-1} s^{-1}) by canopy openness (8.8 % to 90.1 %) of ‘iliahi seedlings 12 months after planting.

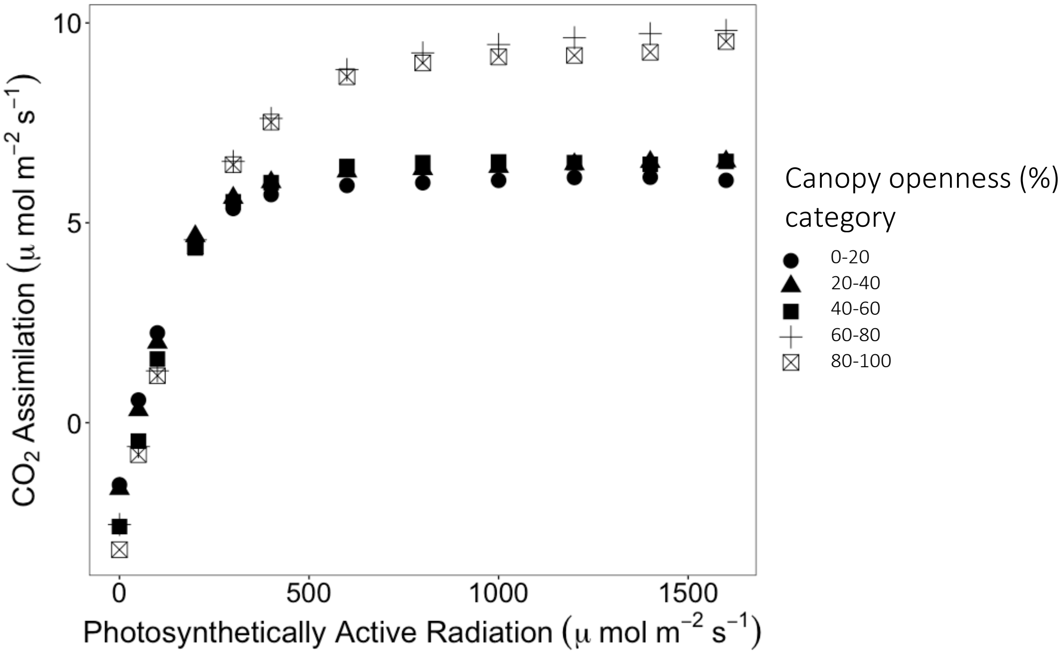


Figure 3.4 Mean light response curves for five canopy openness categories: 0 - 20, 20 - 40, 40 - 60, 60 - 80, 80 - 100 %, n = 16.

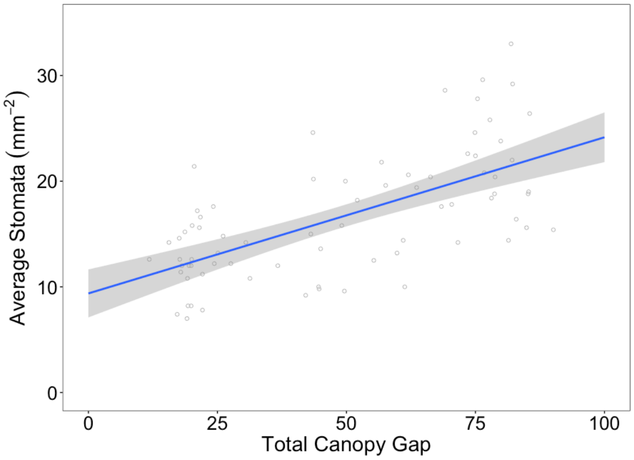


Figure 3.5 Stomatal density (mm^{-2}) on the abaxial (i.e., lower) leaf surface by canopy openness (8.8 % to 90.1 %) of ‘iliahi seedlings 12 months after planting.

3.5 Discussion

Our research demonstrates that ‘iliahi (*Santalum paniculatum*), a hemiparasitic tree, can be successfully underplanted in an established reforestation stand of koa (*Acacia koa*) trees as indicated by overall high survival and growth rates. Variation in canopy openness due to overstory tree mortality provides opportunities to plant ‘iliahi under conditions that favor rapid early growth (Figure 3.1). Koa stands let in a fair amount of light due to the relatively sparse canopy structure and low leaf area index (Pearson and Vitousek 2001; Denslow et al 2006; Scowcroft et al. 2008). This could help to explain high survival across canopy openness. Nevertheless, our results suggest that higher light environments facilitate greater ‘iliahi growth (Figure 3.2). To compare these results to a control (i.e., ‘iliahi not underplanted), we can tentatively draw comparisons to Chapter 2 results where ‘iliahi seedlings of a similar size were planted simultaneously with koa seedlings at different distances only 250 m away from this underplanting study. Mean survival, height, and diameter were higher for underplanted ‘iliahi seedlings from this experiment than for ‘iliahi seedlings simultaneously planted with koa seedlings across distances that had average of 78 % survival, 79 cm height, and 9.6 mm diameter. The higher survival and growth of underplanted ‘iliahi compared to ‘iliahi and hosts planted simultaneously support underplanting as a silvicultural technique for a hemiparasitic tree.

Research with *Santalum album* suggested shade is beneficial for early survival (Barrett and Fox 1994). Barret and Fox (1994) hypothesized this was from less water loss due to reduced leaf temperature, transpiration, and soil evaporation. Our results, however, showed a linear growth response and physiological response with canopy openness. This suggests the early benefit of underplanting may be due more to the presence of an abundant and relatively uniform host root network despite the presence of stem and canopy gaps in our planted stand. Underplanting within a well-established stand of koa seems to mitigate the tradeoff between increased light availability in higher canopy openness and resources obtained via root availability. ʻIliahi, therefore, performed like most tree species by increasing growth with increasing light availability (Walters and Reich, 1996; Sevillano et al. 2016; Ito et al. 2017; Rose et al. 2019). Koa is a notably fast-growing Hawaiian forest species (Scowcroft et al. 2007; Baker et al. 2009), which may rapidly provide a large and uniform enough root network for haustoria connections. Additionally, root hemiparasites are strong below-ground competitors but appear to be relatively weak competitors for light (Atsatt & Strong 1970; De Hullu 1984; Mathies 1995). With parasitic root connections, we hypothesize that the limiting factor for ʻiliahi seedling growth when underplanted shifted from below-ground resources to primarily light.

Physiology measurements also followed a linear response to canopy openness, with higher light compensation, light saturation, and stomatal density under increased canopy openness. This demonstrated plasticity could be beneficial for ʻiliahi to acclimate to varying regeneration settings. Plasticity can also promote survival under environmental changes, in particular for sub-tropical and tropical broadleaf evergreen trees (Lusk et al. 2008; Cavender-Bares and Ramírez-Valiente 2017). Underplanted ʻiliahi from this experiment had similar A_{net} (µmol $m^{-2}s^{-1}$) to ʻiliahi surveyed at a lowland dry forest site (Sandquist and Cordell, 2007). Detection of stomata only on the abaxial leaf surface aligns with results of Stemmermann (1980), who reported similar pattern for several ʻiliahi species and varieties. *Santalum album* growing in Western Australia also only had abaxial leaf surface stomata; however, in contrast to our results, there was significantly greater stomatal density in shaded treatments (Barrett and Fox 1994). Our growth and physiology results support other studies of successful seedling establishment in tropical dry forest canopy gaps likely due to ameliorated dry conditions compared to completely open conditions (Lieberman and Li 1992; Teketay 1997; Cordell et al. 2002; Yelenik et al. 2015). We also want to acknowledge that this study coincided with comparatively wet dry seasons.

There are many other potential contributing factors to underplanting success, such as the age and development of the host canopy. In Western Australia, *S. spicatum* was successfully established beneath 1- to 2-year-old *A. acuminata* (Brand et al. 2000). With a much younger host, the host canopy effects and host root development could become the primary limiting factor for establishment of planted seedlings. Naturally regenerated koa stands reached peak leaf area index around the age of the established koa in this study (Pearson and Vitousek 2001); therefore, koa hosts older than 10 years (as in our study at time of planting ‘iliahi) likely would not alter the observed effect on the planted seedlings as much as younger hosts. The reasons for koa mortality in this stand are unknown. Drought was most likely a factor given the dry period during the year the koa were planted. These rocky soils likely vary in stoniness and depth, which would affect water-holding capacity and thus susceptibility to drought. Another approach to establishing ‘iliahi under koa could be pre-commercial thinning to improve productivity of selected koa trees (Scowcroft et al. 2007; Idol et al. 2017) and increase light availability to subsequently underplanted seedlings. This approach, however, requires determining harvesting logistics and likely a group selection harvest to reduce potential damage.

3.6 Conclusions

Similar to results with *S. album* (Das 2021), ‘iliahi appears to grow well under partial shade during the establishment phase of regeneration. Two years after planting, our results indicate that relatively low canopy openness may be suitable to establish ‘iliahi, yet higher canopy openness promotes early growth. It will be interesting to follow whether ‘iliahi can tolerate shade in later establishment stages, as does *S. album* (Das 2021). This experiment illustrates that underplanting is a viable approach to regenerating ‘iliahi and diversifying initial reforestation efforts. These results contribute to a better understanding ‘iliahi establishment within gaps of established hosts and help improve silvicultural efforts to restore functionally compatible and diverse native dry forests in Hawai‘i.

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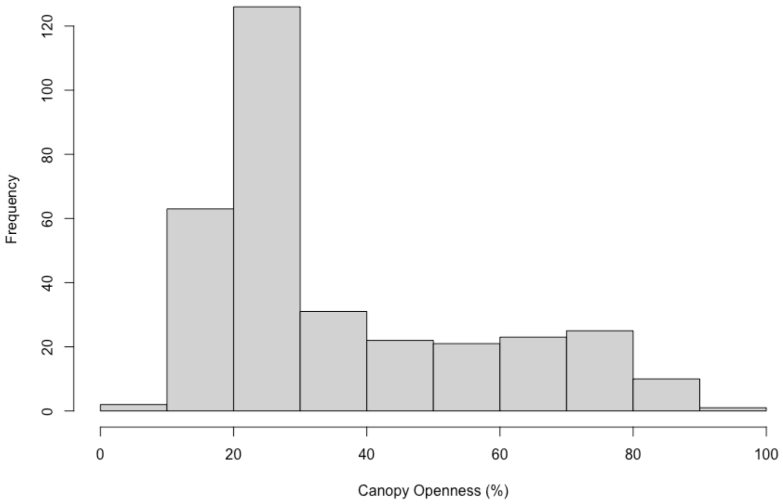
3.9 Appendix 3

Appendix 3.1 Histogram of canopy openness data.



Chapter 4 - Hemiparasitic Resource Transfer Between ‘Iliahi (Hawaiian Sandalwood, Santalum paniculatum) and a Nitrogen-Fixing Host

4.1 Abstract

Trees have many biotic interactions that affect resource acquisition, including root hemiparasitism. Root hemiparasitic trees are capable of photosynthesis yet also gain resources from other plants through root connections called haustoria. We experimentally examined nitrogen and carbon transfer using ^{15}N and ^{13}C stable isotope tracers between the hemiparasite ‘iliahi (*Santalum paniculatum*) and a nitrogen-fixing host, koa (*Acacia koa*), both important forest trees endemic to the Hawaiian Islands that co-occur in dry forests. We split the roots of ‘iliahi and koa between experimental units comprised of either two or three pots. The 2-pot and 3-pot units allowed for the segregation of root systems to examine the possibility of resource transfer via root exudate, and resource transfer via haustoria connecting ‘iliahi and koa. We found that both koa and ‘iliahi were able to translocate ^{15}N laterally through their root system providing an indirect pathway for resource transfer. Detection of ^{15}N in the media of 2-pot units highlighted the potential importance of recognizing and accounting for other mechanisms of resource transfer in hemiparasitic relationships. One-way transfer of ^{15}N from the host to the hemiparasite was detected in leaves and roots, but two-way transfer from the hemiparasite to the host was not detected. One- and two-way transfer of ^{13}C were detected in roots; however, we are cautious as to the biological significance of this ^{13}C result. These experimental results contribute to our understanding of hemiparasitic resource transfer and can help facilitate efforts to restore this unique relationship in native Hawaiian forests.

4.2 Introduction

Plant interactions play a crucial role in plant community dynamics of terrestrial ecosystems such as forests. Interactions can be positive, e.g., facilitative or symbiotic (Michalet and Pugnaire, 2016) or negative, e.g., competitive and parasitic (Matías and Jump, 2012; Těšitel, 2016). Resource acquisition in plants is affected by interactions that impact carbon allocation at the individual and ecosystem level (McNickle and Dybzinski, 2013; Schwinning and Kelly, 2013). Compared to competitive relationships where both interacting individuals are negatively affected, parasitism is expected to impact the host proportionally more. While obligate parasites (holoparasites) are fully dependent upon a host for resources and cannot photosynthesize, hemiparasites can be less dependent for resources and can photosynthesize (Bell and Adams, 2011; Matthies, 2017; Těšitel et al., 2010).

Santalum spp. (sandalwoods) in the Santalaceae family are a prominent example of root hemiparasite trees (Teixeira da Silva et al., 2016). Despite parasitism evolving independently numerous times in angiosperms, host resources are consistently transferred to the parasite via specialized structures called haustoria (Barkman et al., 2007). Resource transfer via haustoria depends on specific anatomical connections, which in turn affect resource transfer mechanisms (Těšitel, 2016). Often the pathway of resource transfer between a host and hemiparasite is viewed as a single one-way path from the host to hemiparasite. There is evidence, however, that resources could also flow from the hemiparasite to the host; two-way transfer has been observed for both nitrogen and phosphorus in *S. album* (Lu et al., 2013; Rocha et al., 2015). If two-way transfer does occur, then the hemiparasitic relationship should be viewed even more dynamically with the host potentially gaining resources from the hemiparasite. There are anecdotal accounts of santalol compounds in hosts that only *Santalum* spp. produce, which may benefit the host and forest community holistically. In addition to transfer via haustoria, resources can be directly transferred to the parasite via mycorrhizal connections (He et al., 2009; Li and Guan, 2008) or indirectly through mechanisms such as decomposition of plant tissues, leaf leachates, fine root turnover, and root exudates (Coskun et al., 2017; Davies and Graves, 1998; Høgh-Jensen, 2006; Press, 1989).

Nitrogen has been reliably identified as an important resource that hemiparasites acquire from their hosts (Cameron and Seel, 2007; Jiang et al., 2010; Lu et al., 2014; Radomiljac et al., 1998). In controlled nursery experiments, host-derived nitrogen has been found to be predominant source of nitrogen in the parasite (Pageau et al., 2003; Tennakoon et al., 1997b). Several other mineral nutrients such as phosphorus, potassium, calcium, sodium, and copper appear to also be transferred between *S. album* and *S. spicatum* A.DC. (Australian sandalwood) and hosts when a hemiparasitic connection is made (Radomiljac et al., 1999a; Struthers et al., 1986) (Radomiljac et al. 1996). Given the importance of acquiring nitrogen from their hosts, nodulated nitrogen-fixing plants have often been identified as preferential hosts for parasites (Lu et al., 2014; Radomiljac et al., 1999b; Veillon and Jaffré, 1995; Woodall and Robinson, 2003). Lu et al. (2013) found that host nodulation resulted in approximately six times more nitrogen transferred to Indian sandalwood (*S. album*) than from a non-nodulated nitrogen-fixing host.

Since hemiparasites photosynthesize, the possibility of photosynthate transfer from the host is interesting, especially as compared to holoparasities that cannot photosynthesize and entirely

rely on hosts for essential resources (Těšitel, 2016). Hemiparasites are reported to photosynthesize at comparable rates to non-parasitic plants, potentially rendering host-derived carbon as more of a backup resource (Těšitel, 2016). Yet in some hemiparasitic relationships, carbon compounds have been acquired from hosts (Irving and Cameron, 2009; Loveys et al., 2001; Radomiljac et al., 1999a; Těšitel et al., 2010). Several studies have shown that some organic components move from the host to hemiparasitic *S. album* and *S. acuminatum* (Loveys et al., 2001; Radomiljac et al. 1998, 1999). Since *S. album* and *S. acuminatum* haustoria appear to only connect to hosts' xylem tissue, any carbon transfer from hosts is likely in the form of readily metabolized sugars and organic acids rather than photosynthates (Ouyang et al., 2015; Pate, 2001; Tennakoon et al., 1997b; Tennakoon and Cameron, 2006).

Although six of the 24 currently recognized *Santalum* spp. are endemic to Hawaiʻi (Harbaugh et al., 2010), where it is known as ʻiliahi or Hawaiian sandalwood, scientific literature on *Santalum* spp. almost exclusively consists of research with *Santalum* spp. from other parts of the Pacific (e.g., *S. album*, *S. acuminatum*, and *S. spicatum*). The endemic diversity, distinct Hawaiian biogeochemistry, and increasing desire to regenerate ʻiliahi for ecological restoration and commercial use, makes it critical to better understand how hemiparasitism manifests in Hawaiian forests. Additionally, in a review on parasitic plants, Těšitel (2016) posited that the uniqueness of parasitic plants makes it imperative to study specific species, as unexpected natural processes may be revealed. For this study, we worked with *Santalum paniculatum* Hook. & Arn.,ʻiliahi endemic specifically to Hawaiʻi Island and currently the only species of ʻiliahi commercially harvested (Braun et al., 2014). As the host, we selected *Acacia koa* A. Gray (koa), also endemic to the Hawaiian Islands, which has been identified as a suitable nitrogen-fixing host for ʻiliahi (Chapters 2 and 3).

Our overall objective was to examine resource transfer within ʻiliahi's hemiparasitic relationship. To address this objective we hypothesized (i) ʻiliahi will perform better when paired with the host koa than when grown independently; (ii) koa will perform better when grown independently than when paired with the hemiparasite ʻiliahi; (iii) when either ʻiliahi or koa have root systems split between two containers resources will not be transferred from one container to another; (iv) when ʻiliahi and koa are growing together, we expect a transfer of resources from koa to ʻiliahi; (v) when ʻiliahi and koa are growing together, we also expect a transfer of resources from ʻiliahi to koa. To test our hypotheses, we used an experimental design that splits and segregates hemiparasite root systems, host root systems, and parasitized root systems (combination of hemiparasite and host roots) to allow for a more controlled examination of resource transfer between the hemiparasite and host. This container design resulted in segregated plant root systems to which stable isotope tracers (^{15}N and ^{13}C) could be applied. We traced nitrogen and carbon transfer in both the media and seedling tissues by using stable isotope tracers to test root exudate, one-way haustoria transfer, and two-way haustoria transfer of nitrogen and carbon.

4.3 Materials and Methods

4.3.1 Experimental site

This experiment was conducted at Kealakekua Forest Nursery (N 19.5084°, W -155.8548°) within the ahupuaʻa[1] of Kealakekua in the Kona moku[2] of Hawaiʻi mokupuni[3] on the slopes of Maunaloa[4], 900 m above sea level. The nursery is in an area considered lower montane dry forest (Asner et al., 2005), with Kona wet season (summer) average high/low temperatures of 28.3/21.1 °C and Kona dry season (winter) average high/low temperatures of 24.6/10.1 °C (U.S. Climate Data, 2021). The experiment was conducted in a semi-controlled environment on a single nursery bench orientated lengthwise North to South. The nursery has a clear polycarbonate catchment roof that provides approximately 40 % of ambient light. Given the steep topography of Maunaloa and the forest surrounding the nursery, most sunlight enters the nursery midday. There is typically afternoon cloud cover and northerly wind; however, the winds are minimized by being on the leeward (i.e., western) side of Maunaloa.

4.3.2 Plant material

This experiment was conducted with two endemic Hawaiian plant species, the hemiparasite, ʻiliahi (*Santalum paniculatum*), and the host, koa (*Acacia koa*). Twelve-month-old ʻiliahi and three-month-old koa container seedlings were obtained from Kealakekua Mountain Nursery (Kealakekua, Hawaiʻi) with a local leeward Hawaiʻi Island open-pollinated seed source. Seedlings were sorted and the most uniform seedlings were selected. Descriptive information about the seedling stock used is provided in Table 4.1. All koa seedlings during nursery seedling production were inoculated with *Bradyrhizobia* spp. to ensure uniform formation of nitrogen-fixing nodules.

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[1] ahupuaʻa: land division usually extending from the uplands to the sea (Ulukau, 2022)
[2] moku: district (Ulukau, 2022)
[3] mokupuni: island (Ulukau, 2022)
[4] Maunaloa: one of five shield volanoes comprising Hawaiʻi Island

Large nursery containers (MT49, 10×10×24 cm, 1589 mL, Stuewe & Sons Inc., Tangent, OR, USA) were joined together to create experimental units. Either two or three containers were used to create "2-pot" and "3-pot" experimental units, respectively (Figure 4.1). The containers were filled with a 3:2 mix of potting soil (Sunshine® Mix #4 Aggregate Plus with Mycorrhizae) and perlite (Therm-o-rock) as well as 12.8 mg/ml of 90 day controlled-release fertilizer (13-11-11, Nutricote® Total), 6.4 mg/ml ironite (1-0-1, Pennington), and 4.0 mg/ml triple superphosphate (0-45-0, Simplot). All containers were filled with the same mass of media that was then compacted to the same volume, resulting in similar soil bulk densities.

Table 4.1 Species, age (months since seed germination), type of nursery container, mean initial height (cm) (± SE), mean initial diameter (mm) (± SE), mean root:shoot ratio (± SE), and mean root volume (cm^3) (± SE) of the two species used in the experiment: ʻiliahi and koa, n = 15 per species. AirCell 105 are rectangular nursery containers with a volume of 105 mL (BCC, Landskrona, Skåne, Sweden).

Species	**Age (months)**	**Nursery container**	**Initial height (cm)**	**Initial diameter (mm)**	**Root:shoot ratio**	**Root volume (cm^3)**
ʻiliahi	12	Air Cell 105	23 (1)	4.1 (0.1)	0.68 (0.06)	16 (1)
koa	3	Air Cell 105	21 (1)	2.4 (0.1)	0.32 (0.02)	19 (1)

In April 2021, the original growing media was carefully removed from the seedling roots, and their root system was segregated ("split") into two halves. Seedlings were immediately transplanted into the units described above. One seedling was transplanted into the 2-pot units (root system split between the two containers) and two seedlings (one ʻiliahi and one koa) were transplanted into the 3-pot units, each seedling with a split root system and with the middle container containing the roots of both species (Figure 4.1). While the seedling stem base rested on the junction between two containers, each half of the root system was transplanted into the adjacent container. This container design resulted in spatially segregated roots systems to explore resource transfer pathways.

The 2-pot and 3-pot units and overall experimental design were tested during a preliminary study and seedlings produced haustoria within six months. For this experiment, after six months we did not have haustoria; therefore, we grew the seedlings for 12 months (i.e., until April 2021) to ensure haustoria formation. Throughout the grow-out stage of the 2-pot and 3-pot units, koa had a faster growth rate than ‘iliahi; therefore, koa shoots were occasionally trimmed for manageability. Any trimmed shoots were dried, weighed, and incorporated into final shoot biomass. The 2-pot and 3-pot units were watered to field capacity regularly and there was no additional fertilization. The units were placed randomly on the nursery bench and were rotated every three months to reduce potential spatial effects within the nursery bench.

Only 3-pot units with large haustoria were used in the experiment. Initially, 40 3-pot units were established. After 12 months, only 34 were considered viable for stable isotope tracer application. Of the 3-pot units destructively sampled (n = 34) at the end of the experiment, 82.4% (n = 28) had haustoria and 32.4% (n = 11) had large (i.e., >5 mm) haustoria. We assumed larger haustoria development would have greater detection potential; therefore, each treatment was reduced to a consistent sample size of five, yielding 20 total experimental units. In addition to confirming haustoria development, nitrogen-fixing nodules were confirmed on all koa used in the experiment.

4.3.3 Treatments

Pre-stable isotope tracer application media and roots were collected prior to stable isotope tracer application from extra units since media and root sampling is destructive. Approximately 80 mL of media was collected and inspected to ensure no roots remained in the media. Root samples were a representative subsample of the root systems. Leaves were collected prior to stable isotope tracer application for analysis and consisted of 2-3 fully developed leaves from the upper one-third portion of a seedling.

The ^{15}N solution was made by mixing 0.16 g of 98 atom % $^{15}NH_4{}^{15}NO_3$ (MilliporeSigma, Burlington, MA, USA) and 0.24 g of non-labeled NH_4NO_3 (MilliporeSigma, Burlington, MA, USA) with 1 liter of warm water. Next, 100 mL of $^{15}NH_4NO_3$ solution was applied evenly across the surface of the applicant container of each experimental unit. To apply ^{13}C, we followed a similar process as Norris et al. (2012). The applicant plant was fully enclosed in an airtight sealed plastic bag (33 × 38.1 cm for ‘iliahi at 2.6 mil thickness, 50.8 × 101.6 cm for koa at 2 mil thickness) with a 2-ply reinforced duct tape patch between 10:00 and 14:00 hours. The patch was injected with a 22-gauge needle (3.81 cm length) and syringe containing 70 mL of 99 atom % $^{13}CO_2$. After the $^{13}CO_2$ injection, another layer of duct tape was applied to ensure an airtight seal, and the seedling remained enclosed for two hours to promote $^{13}CO_2$ assimilation. Before removing the bag from the applicant plant, the recipient plant was fully enclosed in an airtight sealed bag to minimize the potential assimilation of residual $^{13}CO_2$ before it dissipated. The experiment concluded ten days after stable isotope tracers were applied and final seedling measurements were taken.

The 2-pot units (Treatments 1 and 2) either had ‘iliahi or koa, respectively, growing alone with split roots (Figure 4.1a-b). To evaluate if ^{15}N root exudate transfer occurred, ^{15}N stable isotope tracer was applied to one container of the 2-pot unit and a post-application media sample was taken from the second container that the ^{15}N was not applied to. The 3-pot units (Treatments 3 and 4) had both ‘iliahi and koa growing together with split roots in a shared container (Figure 4.1c-d). To test and evaluate the movement of resources between seedlings, ^{15}N and ^{13}C were applied to one of the two plants in a 3-pot unit, known as the “applicant” plant. The “recipient” plant was the other seedling in the 3-pot unit and to whom the stable isotope tracer may potentially be transferred to. Treatment 3 evaluated one-way transfer with the host koa as the applicant plant and the hemiparasite ‘iliahi as the recipient plant. Treatment 4 evaluated one-way transfer with the hemiparasite ‘iliahi as the applicant plant and the host koa as the recipient plant. Together, treatments 3 and 4 tested whether two-way transfer occurred.

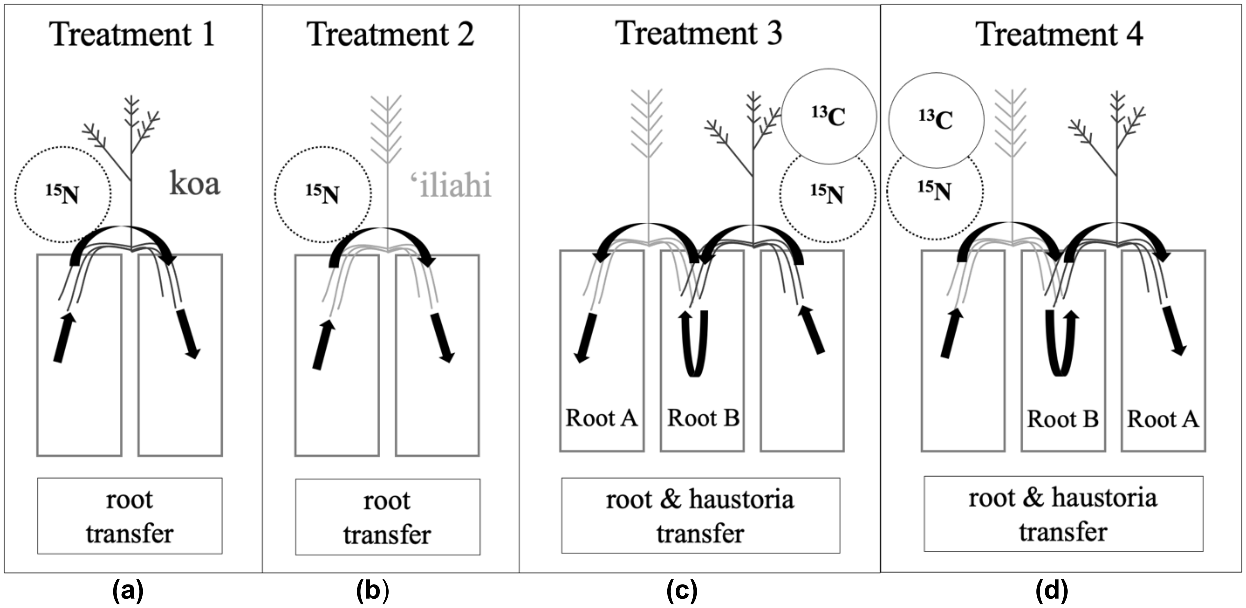

Figure 4.1 The four treatments with the hemiparasite, ‘iliahi, and the host, koa, **(a)** 2-pot koa **(b)** 2-pot ‘iliahi **(c)** 3-pot with isotopes (^{15}N and ^{13}C) applied to the host, koa, (applicant) and the hemiparasite as the recipient **(d)** 3-pot with isotopes (^{15}N and ^{13}C) applied to the hemiparasite, ‘iliahi, (applicant) and the host as the recipient. Arrows show the potential transfer of resources from the applicant to the recipient. Treatments 1 and 2 examine lateral redistribution and root exudation into media. Treatments 3 and 4 examine lateral redistribution and pathway through haustoria. Root A are recipient roots from the container with only recipient roots. Root B are recipient roots from the center container that had been associated with the applicant plant roots.

4.3.4 Sampling and measurements

Ten days after isotope application, seedling root collar diameter (RCD) and height (root collar to the apical meristem) were measured, and tissue samples collected. All seedlings were destructively sampled and separated into leaves, stems, and roots. Leaf water potential was measured with a pressure chamber (Model 600D, PMS Instrument Company, Albany, OR, USA). ʻIliahi has short petioles that cannot be inserted into a pressure chamber; therefore, we sampled the terminal leaves/non-lignified stem. Since koa is heteroblastic with two 'leaf' forms (Rose et al., 2019), we only sampled the typically earlier-form bipinnately compound, horizontally oriented true leaves instead of the phyllodes that emerge later in development. Two to three fully developed leaves from the upper one-third portion of the seedling stems were collected. Remaining leaves were separated from the stem and dry weights were added to those of the foliar sample leaves for total leaf weight. The stem was separated from the roots at the root collar and added to total leaf weight for total shoot weight. Total shoot mass was comprised of pre- and post-application leaves, remaining leaves, and stems.

Roots in each container were measured separately. Roots from the 3-pot center container where parasitized roots grew were carefully separated by species (ʻiliahi and koa). Recipient plant roots from the center container that had been associated with the applicant plant roots are referred to as Roots B, while Roots A refer to the recipient roots from the container with only recipient plant roots (Figure 4.1). Post-application root samples were a representative subsample of the root systems. For parasitized roots from the center-container, the number of haustoria were counted, presence of large haustoria (>5mm) were noted, and weighed with ʻiliahi roots. For all koa roots the presence of nodules were noted. Root volume was quantified using water displacement in a graduated cylinder. Total root mass was the combined mass of each species across all segregated root systems. ^{15}N and ^{13}C were considered detected in leaf or root samples when the δ^{15}N or δ^{13}C post-application for leaf or root samples were greater than the pre-application for leaf or root samples.

All sampled materials were dried at 60°C for 48 h and then weighed. Dried samples specifically identified for analysis were composited, pulverized, and encapsulated at the University of Hawaiʻi at Hilo Analytical Lab. Foliar nutrients (P, K, Ca, Mg, S) were analyzed at the University of Hawaiʻi at Hilo Analytical Lab. Stable isotope samples were analyzed at the University of California at Davis Stable Isotope Facility with an EA-IRMS system. δ^{15}N was compared to an air constant (atmospheric N_2; primary reference for measurements of nitrogen isotopes) while

$\delta^{13}C$ was compared to the VPDB constant (Vienna PeeDee Belemnite; primary reference for measurements of carbon isotopes). Throughout work with the stable isotope tracers care was taken with applying, collecting, storing, and processing samples to ensure there was no cross-contamination.

4.3.5 Experimental design and statistical analyses

Each of the four treatments were arranged in a completely randomized experimental design. All data was analyzed in R software (R Core Team, 2022). Seedling performance response variables were analyzed separately by species using a general linear model with type of unit (2-pot or 3-pot) as the fixed factor. Foliar water potential was analyzed with a general linear model with species as the fixed factor. Seedling foliar nutrient data were analyzed separately by species with a multivariate analysis of variance (MANOVA) with type of unit as the fixed factor. Stable isotope media data was analyzed with a two-way analysis of variance (ANOVA) with species and application (i.e., pre- and post- application) as the fixed factor. Stable isotope foliar and root data were analyzed with a one-way ANOVA with application as the fixed factor. Outlier tests were run for all stable isotope data. A significant outlier was detected for media and foliar post-application $\delta^{15}N$ data. There were no significant co-variates or correlations to account for the identified outliers.

Residuals from all response variables were tested to ensure normality and homogeneity of variance assumptions. Media, foliar, and root $\delta^{15}N$ did not meet assumptions, therefore, these data were log(1+x) transformed. If a significant treatment effect was detected ($p \leq 0.05$), Tukey's HSD test was used to test pairwise comparisons with $\alpha = 0.05$.

4.4 Results

4.4.1 Seedling performance

Height, diameter, root, and shoot measurements were not significantly different between individuals in the 2-pot and 3-pot units for either species (Table 4.2; Appendix 4.1). Leaf water potentials were less negative for koa than ʻiliahi ($F_{2,59}=33.12$, $p < 0.001$).

For ʻiliahi foliar nutrients, only sulfur (S) was greater in 3-pot than 2-pot units ($F_{1,8}=6.57$, $p = 0.034$). By contrast, foliar phosphorous (P) ($F_{1,8}=8.31$, $p = 0.020$), potassium (K), calcium (Ca) ($F_{1,8}=9.38$, $p = 0.016$), and magnesium (Mg) were lower in 3-pots than 2-pot units. For koa foliar nutrients, P ($F_{1,8}$=7.10, p = 0.029), K, Mg ($F_{1,8}$=12.84, p = 0.007), and S were less in 3-pot than 2-pot units.

Table 4.2 Mean (± SE) final height (cm), root collar diameter (mm), total root mass (g), total shoot mass (g), and foliar water potential (MPa) at the end of the experiment (i.e., 12 months after the start of the experiment, April 2022) for the hemiparasite, ʻiliahi, and the host, koa, in 2-pot and 3-pot experimental units, n = 5. There were no significant differences between 2-pot and 3-pot units for either species.

Species	Type of Unit	Height (cm)	Diameter (mm)	Total Root mass (g)	Total Shoot mass (g)	Foliar Water Potential (MPa)
ʻiliahi	2-pot	53 (4)	6.4 (0.3)	3.1 (0.6)	9.1 (1.3)	-2.2 (0.1)
	3-pot	73 (12)	6.8 (0.4)	5.6 (1.2)	12.4 (2.2)	-2.1 (0.1)
koa	2-pot	116 (6)	10.2 (0.6)	27.4 (3.9)	57.9 (7.2)	-1.3 (0.1)
	3-pot	124 (8)	10.9 (0.8)	44.5 (7.1)	62.7 (8.1)	-1.3 (0.1)

Table 4.3 Mean (± SE) nutrient concentration (%) for ʻiliahi (Santalum paniculatum) and koa (Acacia koa) in both 2-pot and 3-pot experimental units. Nutrients include: phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S). Within each nutrient parameter, different letters indicate significant differences (α = 0.05) between 2-pot and 3-pot units, n = 5.

Species	Type of Unit	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
ʻiliahi	2-pot	0.88 (0.08) a	2.40 (0.19) a	1.55 (0.15) a	0.34 (0.03) a	0.21 (0.02) a
	3-pot	0.59 (0.06) b	2.19 (0.08) a	0.77 (0.20) b	0.29 (0.03) a	0.29 (0.03) b
koa	2-pot	0.64 (0.09) A	1.51 (0.28) A	0.61 (0.04) A	0.22 (0.01) A	0.19 (0.03) A
	3-pot	0.38 (0.05) B	1.26 (0.13) A	0.63 (0.10) A	0.17 (0.01) B	0.16 (0.04) A

4.4.2 Resource transfer

For both ʻiliahi and koa 2-pot units, media of the container that $^{15}NH_4{}^{15}NO_3$ was not applied to, had higher post-application $\delta^{15}N$ than pre-isotope media ($F_{2,17}$ = 11.78, p = 0.002; Table 4.4; Appendix 4.2). Furthermore, post-application $\delta^{15}N$ was higher for koa media than for ʻiliahi media.

Table 4.4 Mean (± SE) $\delta^{15}N_{Air}$ (‰) for media samples from 2-pot experimental units. Pre ʻiliahi and koa media samples were collected before stable isotope tracer application. Post ʻiliahi and koa media samples were collected 10 days after stable isotope application from 2-pot container that did not receive the stable isotope, n = 5. Different letters indicate significant differences (α = 0.05).

Species	Application	$\delta^{15}N_{Air}$ (‰)
‘Iliahi	Pre	0.15 (0.63) a
Koa	Pre	-0.74 (0.13) a
‘Iliahi	Post	3.91 (0.66) b
Koa	Post	12.49 (5.49) c

After $^{15}NH_4{}^{15}NO_3$ was applied to the koa media in the 3-pot units, the recipient ‘iliahi had higher foliar $\delta^{15}N$ ($F_{1,8} = 7.46$, $p = 0.029$) and root $\delta^{15}N$ ($F_{2,12} = 5.59$, $p = 0.023$). Both Roots A and B of ‘iliahi appear to be enriched; however only the Roots B in the center container where the haustoria connections formed had a statistically significant enrichment over the pre-measurement (Table 4.5; Appendix 4.3). When $^{15}NH_4{}^{15}NO_3$ was applied to ‘iliahi, koa foliar $\delta^{15}N$ ($F_{1,8} = 2.65$, $p = 0.142$) and root $\delta^{15}N$ ($F_{2,11} = 0.08$, $p = 0.923$) were not higher than pre-application $\delta^{15}N$ (Table 4.5).

Application of $^{13}CO_2$ to either koa ($F_{1,8} = 0.88$, $p = 0.377$) or ‘iliahi ($F_{1,8} = 1.17$, $p = 0.311$) did not increase the foliar $\delta^{13}C$ of the recipient plant (Table 4.5; Appendix 4.4). This contrasted with roots, where after $^{13}CO_2$ was applied to either koa or ‘iliahi, the $\delta^{13}C$ was higher in target roots post application for both koa ($F_{2,12} = 4.70$, $p = 0.031$) and ‘iliahi ($F_{2,11} = 7.05$, $p = 0.011$), but only in the roots of the center container where haustoria connections formed (Table 4.5).

Table 4.5 Mean (± SE) $\delta^{15}N_{Air}$ (‰) and $\delta^{13}C_{VPDB}$ (‰) for foliar and root samples from 3-pot units. Pre foliar and root samples were collected before stable isotope application from the recipient plant. Post foliar and root samples were collected 10 days after application from the recipient plant. Post roots include roots from the container with parasitized roots (Post root B) and roots from the container with only recipient plant roots (Post root A), n = 5. Different letters indicate significant differences ($\alpha = 0.05$).

Isotope tracer	**Tissue**	**Application**	**Treatment 3** Recipient = ‘iliahi	**Treatment 4** Recipient = koa
$\delta^{15}N_{Air}$ (‰)	**Foliar**	**Pre**	0.79 (0.26) A	-1.09 (0.29) a
	Foliar	**Post**	18.11 (14.04) B	-1.80 (0.34) a
	Root	**Pre**	-0.35 (0.74) A	0.15 (0.93) a
	Root B	**Post**	5.13 (2.31) B	0.20 (0.61) a
	Root A	**Post**	3.28 (1.17) AB	0.07 (0.93) a
$\delta^{13}C_{VPDB}$ (‰)	**Foliar**	**Pre**	-29.71 (0.74) A	-29.81 (0.31) a
	Foliar	**Post**	-28.43 (1.15) A	-29.51 (0.27) a
	Root	**Pre**	-27.41 (0.50) A	-28.68 (0.15) a
	Root B	**Post**	-25.69 (0.33) B	-23.70 (1.19) b
	Root A	**Post**	-26.59 (0.34) AB	-28.02 (0.61) a

4.5 Discussion

Plant biotic relationships have many interactions, and for hemiparasites, there is an additional interaction through haustoria connections. Our experimental results support root transfer from root exudates and, most likely, one-way haustoria transfer of nitrogen between the hemiparasite ‘iliahi and host koa. In this experiment, carbon transfer was also detected, but only in root tissues. There was no effect of treatments on either ‘iliahi or koa performance response variables, yet there were some significant foliar nutrient differences.

Hypothesis (iii) that there would be no detectable resource transfer in 2-pot units was unsupported because ^{15}N was detected in the 2-pot container that did not receive the ^{15}N application. While such detection was unexpected, the transfer of resources through root exudation adds a layer of complexity that alters the assumptions of hemiparasite resource transfer. Furthermore, post-application koa 2-pot had higher $\delta^{15}N$ than ‘iliahi 2-pot (Table 5.4). This could be due to koa having a larger root mass than ‘iliahi (Table 5.2) and, therefore, more surface area for root exudation. The higher media $\delta^{15}N$ could also be due to other species differences regarding rhizodeposition such as proportion of fibrous root tips, presence of nitrogen-fixing nodules, or the microbial community (Kušliene et al., 2014). While haustoria most likely have a key role in hemiparasitic transfer, the 2-pot unit provided a method to examine other pathways within hemiparasitic relationships, such as root exudate transfer. This knowledge is essential in understanding hemiparasitic transfer and raises caution for hemiparasite stable isotope tracer experiments. Lu et al. (2013) noted that their experimental design could not account for root transfer, whether from root exudates or deposition. Whereas Loveys et al. (2001) purposefully included a treatment to attempt to confirm whether detected ^{14}C transfer was from haustoria or root exudate, which ^{14}C was not detected from root exudate uptake.

The nitrogen results contribute to our confidence that haustoria transfer was likely occurring given the magnitude of difference between pre- and post-application ʻiliahi foliar results when ^{15}N was applied to koa (Table 5.5). This result is similar to experiments with other *Santalum* spp. that have detected ^{15}N transfer from hosts (Lu et al., 2020, 2013; Tennakoon et al., 1997b). These results also align with experiments with other hemiparasites (Cameron and Seel, 2007; Pageau et al., 2003). There was also significantly higher post-application root $\delta^{15}N$ for ʻiliahi roots from the center container shared with koa roots (Table 5.5). It is reasonable that ʻiliahi roots from the center container would have higher $\delta^{15}N$ because this container is where haustoria connections formed. ʻIliahi roots in the container with only ʻiliahi roots would have required further $\delta^{15}N$ translocation. It is also possible that given the proximity to koa roots, there was more koa root exudate available to ʻiliahi roots. Acquisition of nitrogen from hosts is likely why hemiparasites are regularly reported to prefer nitrogen-fixing hosts (Brand, 2009; Ouyang et al., 2015; Radomiljac et al., 1998; Tennakoon et al., 1997a; Veillon and Jaffré, 1995). These ʻiliahi foliar and root results partially support hypothesis (iv) that resources would transfer from koa to ʻiliahi because when both species were grown together in 3-pot units and ^{15}N applied to koa, ^{15}N was detected in ʻiliahi foliage and roots, therefore indicating one-way haustoria transfer of nitrogen from the host to the hemiparasite.

Hypothesis (iv) that resources would transfer from koa to ʻiliahi also included carbon transfer, and the carbon results were obscure. ^{13}C was not detected in ʻiliahi leaves, but it was detected in ʻiliahi roots (Table 5.5). Even with this statistical significance, however, we are hesitant to confirm its biological significance. Part of this hesitancy is because the magnitude of difference between pre- and post-application is less than 1. Since 99 % atom $^{13}CO_2$ was used, a larger magnitude difference, such as for our ^{15}N results was anticipated. Although, since ^{15}N and ^{13}C had different application methods, including length of exposure, it may be misleading to compare magnitudes of difference. A study that applied ^{13}C to different tree species reported foliar $\delta^{13}C$ values around -20 and -25 for their lower tracer application (Glaser et al., 2012). Perhaps the presented $\delta^{13}C$ values were a clear detection of ^{13}C or perhaps longer $^{13}CO_2$ exposure would have increased detection confidence. Differences between our results and two *Santalum* spp. studies that detected carbon transfer could be due to different methods. For example, Lu et al. (2020) applied liquid $^{13}CO(^{15}NH_2)_2$ to six-year-old trees in the field, and Loveys et al. (2001) applied $^{14}CO_2$ to 1-year-old seedlings.

For hypothesis (v) that resources would also transfer from ʻiliahi to koa, there was no detection of ^{15}N transferring from ʻiliahi to koa. This result is contrary to Lu et al. (2013) that presented two-way transfer results for nitrogen between *S. album* and a nitrogen-fixing host (*Dalbergia odorifera* T. Chen). Different results from studies with other *Santalum* spp. could be due to variations among *Santalum* spp. or deviations in methodologies. ^{13}C, however, was detected as transferring from koa to ʻiliahi and from ʻiliahi to koa with post-application koa root B $\delta^{13}C$ (Table 5). If this result is biologically significant, then this result may align with Lu et al. (2020) that detected concurrent carbon and nitrogen transfer for *Santalum album* and its hosts in a field setting. Results from Lu et al. (2020) were contrary to Loveys et al. (2001), who only reported one-way transfer of carbon from the host to the hemiparasite.

Both seedling performance hypotheses (i and ii) that ʻiliahi and koa would perform differently in 2-pot units versus 3-pot units were not supported because there were no differences between 2-pot or 3-pot unit performance response variables for either ʻiliahi or koa. This could be because haustoria took longer than expected to connect during the 12 months prior to labelling treatments. The foliar nutrient results may indicate that ʻiliahi either does not acquire those resources from koa or reduced nutrient concentration in 3-pot ʻiliahi could be due to competition for those resources in the shared 3-pot container. The competition for resources could outweigh any detection of transfer for the nutrients as well as any expected growth differences. Further, 3-pot koa had less nutrient concentration potentially indicating that ʻiliahi acquired those resources from koa either through haustoria or competition. Additional resource transfer, other than nitrogen, has also been identified for other *Santalum* spp. (Radomiljac et al., 1999a; Struthers et al., 1986). ʻIliahi's more negative plant water potential than koa concurs with past results whereby other hemiparasites and sandalwoods maintain lower water potential to cause flow of resources from hosts (Radomiljac et al., 1999b; Sandquist and Cordell, 2007).

In this experiment, our sample size was reduced to five which likely contributed to the high observed variation. Other potential reasons for variation are haustoria biomass, haustoria activity, amount of koa nitrogen-fixation. Results are also likely dependent on the grow-out stage conditions, therefore, implementing different growing conditions and even environmental stressors could be insightful. As such, Tesitel (2016) suggested that host quality and environmental conditions affects photosynthetic activity, which in turn could affect carbon acquisition from hosts. While our experimental design allowed examination of root transfer, it cannot separate root transfer and haustoria transfer. Even with root transfer, haustoria transfer was likely occurring given the large haustoria (> 5 mm), although how much haustoria transfer occurred cannot be determined with this experiment. In addition to being unable to separate haustoria and root exudate transfer with our experimental design, our 3-pot unit could not account for any potential mycorrhizal transfer. There is evidence of ^{15}N isotope tracer transfer through both root exudate and mycorrhizae from a tropical nitrogen-fixing tree (*Gliricidia sepium* (Jacq.) Steud.) to an associated grass (Jalonen et al., 2009). Therefore, mycorrhizal transfer could have also contributed to stable isotope tracer detection.

4.6 Conclusions

ʻIliahi's hemiparasitic relationship is complex and requires continued meticulous investigation. This study examined different types of transfer occurring in a hemiparasitic relationship using a distinct experimental unit design and stable isotope tracers. We are confident about nitrogen transfer to ʻiliahi and the potential for carbon transfer is interesting. The detection of lateral redistribution and root exudation into media highlights that while haustoria are unique to parasitic transfer, other pathways also exist within hemiparasitic relationships and should be taken into consideration. An important future direction is to gain a clearer understanding of haustoria formation and how to encourage haustoria connections. This contribution to hemiparasitic resource transfer may help efforts to regenerate and restore ʻiliahi, other hemiparasitic trees, and the relationship to forest ecosystems.

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4.9 Appendix 4

Appendix 4.1 Mean (± SE) root A mass (g), root B mass (g), stem (g), leaf mass (g), and root:shoot ratio at the end of the experiment (i.e., 12 months after the start of the experiment, April 2022) for hemiparasite ‘iliahi (Santalum paniculatum) and host koa (Acacia koa) in 2-pot and 3-pot experimental units, n = 5. There were no significant differences between 2-pot and 3-pot units for either species.

Species	**Type of Unit**	**Root A mass (g)**	**Root B mass (g)**	**Stem (g)**	**Leaf mass (g)**	**Root:Shoot**
‘iliahi	2-pot	3.1 (1.1)	2.0 (0.4)	5.9 (1.1)	3.2 (0.5)	0.58 (0.05)
	3-pot	1.4 (0.2)	4.8 (1.1)	6.8 (1.2)	5.6 (1.0)	0.46 (0.04)
koa	2-pot	14.0 (1.6)	13.4 (2.6)	28.8 (4.0)	23.8 (4.8)	0.54 (0.05)
	3-pot	27.1 (4.5)	19.4 (4.0)	38.9 (4.3)	27.7 (2.7)	0.76 (0.09)

Appendix 4.2 $\delta^{15}N_{Air}$ (‰) for media samples from 2-pot experimental units. Pre media samples were collected before stable isotope application. Post ʻiliahi and koa media samples were collected 10 days after stable isotope application from the ʻiliahi and koa 2-pot experimental unit container that did not receive the stable isotope, respectively, n=5. Bolded value is the replicate with the outlier $\delta^{15}N_{Air}$ (‰) value.

Media Sample	**Replicate**	**$\delta^{15}N_{Air}$ (‰)**
Pre	1	0.44
Pre	2	0.32
Pre	3	-1.87
Pre	4	-0.22
Pre	5	-0.26
Post ʻiliahi	1	3.45
Post ʻiliahi	2	1.57
Post ʻiliahi	3	4.31
Post ʻiliahi	4	4.91
Post ʻiliahi	5	5.29
Post koa	**1**	**34.04**
Post koa	2	4.16
Post koa	3	7.14
Post koa	4	6.27
Post koa	5	10.83

Appendix 4.3 $\delta^{15}N_{Air}$ (‰) for foliar and root samples from 3-pot experimental units. Pre foliar and root samples were collected before stable isotope application from the recipient plant. Post foliar and root samples were collected 10 days after application from the recipient plant. Post root samples include samples from both sets of roots from the recipient plant. Roots from the container with both applicant and recipient roots (Post root B) and roots from the container with only recipient plant roots (Post root A), n = 5.

Applicant	**Recipient**	**Rep.**	**Number haustoria**	**Pre Foliar**	**Post Foliar**	**Pre Root**	**Post Root B**	**Post Root A**
koa	ʻiliahi	1	21	1.13	3.60	-3.06	1.33	2.25
		2	33	0.70	2.11	0.26	2.34	2.83
		3	22	1.35	5.59	0.65	3.48	2.52
		4	37	-0.14	5.04	1.07	4.35	1.01
		5	**59**	**0.92**	**74.20**	**-0.68**	**14.14**	**7.79**
ʻiliahi	koa	1	5	-0.25	-2.79	0.81	0.26	-0.53
		2	15	-1.52	-0.80	1.46	—	3.06
		3	70	-1.69	-1.64	1.92	-0.28	-1.48
		4	42	-0.58	-2.24	-2.46	-1.03	2.78
		5	17	-1.42	-1.57	0.35	1.86	-0.48

Appendix 4.4 $\delta^{13}C_{VPDB}$ (‰) for foliar and root samples from 3-pot experimental units. Pre foliar and root samples were collected before stable isotope application from the recipient plant. Post foliar and root samples were collected 10 days after application from the recipient plant. Post root samples include samples from both sets of roots from the recipient plant. Roots from the container with both applicant and recipient roots (Post root B) and roots from the container with only recipient plant roots (Post root A), n = 5.

Applicant	**Recipient**	**Rep.**	**Number haustoria**	**Pre Foliar**	**Post Foliar**	**Pre Root**	**Post Root B**	**Post Root A**
koa	‘iliahi	1	21	-29.42	-30.83	-25.67	-26.76	1.33
		2	33	-31.48	-30.17	-27.58	-24.70	2.34
		3	22	-27.51	-28.00	-27.22	-25.72	3.48
		4	37	-28.93	-24.26	-27.84	-25.55	4.35
		5	59	-31.18	-28.86	-28.73	-25.71	14.14
‘iliahi	koa	1	5	-28.98	-29.87	-29.09	-28.32	0.26
		2	15	-29.80	-29.36	-28.13	—-	-0.28
		3	70	-30.39	-30.05	-28.61	-1.48	—
		4	42	-30.15	-29.21	-28.37	2.78	-1.03
		5	17	-29.72	-28.93	-28.65	-0.48	1.86

Chapter 5 - Conclusions

5.1 Dissertation Objectives

This dissertation focused on the regeneration of ‘iliahi (Hawaiian sandalwood, *Santalum paniculatum*) to support restoration and reforestation efforts of Hawaiian tropical dry forests. Three projects were established on Hawai‘i Island on the slopes of Maunaloa at Kealakekua Mountain Reserve to fill knowledge gaps and to improve the survival and establishment of planted ‘iliahi seedlings. Two projects were in the field, and one project was in the nursery. As with many previously dry forested lands in west Hawai‘i, Kealakekua Mountain Reserve was deforested for agriculture and is now dominated by pasture grass. Since ‘iliahi is hemiparasitic, any typical forestry research question has an additional layer of complexity due to this unique relationship in trees. Investigating resource allocation between host plants and the hemiparasitic ‘iliahi contributed insight into complex plant relationships. Hemiparasitic trees are ecologically distinct, and this dissertation research advanced our scientific knowledge of how hemiparasitic trees interact with hosts and influence the ecosystems in which they reside. Specifically, studying resource allocation for hemiparasitic trees contributed to understanding early development and regeneration of the hemiparasite as well as its hosts.

Increased understanding of ‘iliahi as a hemiparasitic tree contributes to restoring tropical dry forests through native species regeneration. Knowledge gained can increase restoration success by matching ‘iliahi with suitable hosts and planting environments. *Santalum paniculatum* is just one of six currently recognized ‘iliahi species. Results from this work could apply to other ‘iliahi and serve as a foundation for studying the other species in a local context. Additionally, results could be relevant to improving our understanding of the biology and ecology of other global *Santalum* spp. and other hemiparasitic plants.

5.2 Summary of Experiments

5.2.1 Host Suitability

In June 2019, 360 1-year-old container ‘iliahi seedlings were planted in a completely randomized experimental design. ‘Iliahi seedlings were randomly assigned one of nine treatments: ‘iliahi with (1) no host (control), (2) koa at <0.2 m, (3) koa at 0.5 m, (4) koa at 1.0 m, (5) koa at 2.0 m, (6) ‘a‘ali‘i at <0.2 m, (7) ‘a‘ali‘i at 0.5 m, (8) ‘a‘ali‘i at 1.0 m, and (9) ‘a‘ali‘i at 2.0 m. After three years, survival was greatest only for ‘iliahi paired with koa at 1.0 m (78 %) compared to the control with no host (53 %) and when paired with koa at 2.0 m (55 %). ‘Iliahi grew more and had greater foliar nitrogen concentration when paired with koa (*Acacia koa*) at a close distance than with aʻaliʻi (*Dodonaea viscosa*) at any distance or the control with no host. Specifically, ʻiliahi growth was greatest when paired with koa at <0.2 m distance. For sites with few or no pre-established hosts, such as abandoned pastures, ʻiliahi growth can be enhanced by co-planting in proximity to koa.

5.2.2 Underplanting

In August 2020, 324 9-month-old container ʻiliahi seedlings were planted under the canopy of 10-year-old koa (*Acacia koa*) trees. Canopy openness above each ʻiliahi seedling varied throughout the planting site with a mean value of 35.9 % and a range of 8.8 % to 90.1 %. The mean distance of ʻiliahi planting sites to the nearest koa was 3.29 m with a range of 0.4 m and 13.0 m. After two years, survival averaged 97.5%. ʻIliahi seedling height and diameter were greater with larger canopy openness, and physiological measurements also increased with greater canopy openness. Though significant, the relationships between canopy openness and the measurements were low to moderate.

5.2.3 3-Pot

In April 2021, 2-pot and 3-pot experimental units were established with the roots of ʻiliahi and koa split between containers. The 2-pot and 3-pot units allowed the segregation of roots to examine root exudate transfer and haustoria transfer between ʻiliahi and koa. In April 2022, ^{15}N and ^{13}C stable isotope tracers were applied to specific units and species. The detection of root exudate transfer of ^{15}N in the media of 2-pot units highlighted the importance of recognizing other mechanisms of ^{15}N transfer than from haustoria connections. One-way transfer of ^{15}N from the host koa to the hemiparasite ʻiliahi was detected in leaves and roots. One- and two-way transfer of ^{13}C were detected in roots; however, we are cautious as to the biological significance of this result.

5.3 Management Implications

Through conversations with practitioners, this research fulfilled identified needs by conducting novel research on ʻiliahi that is directly applicable to on-site management, from resource transfer, to host selection, planting distances, and silvicultural treatments. Research to help fill knowledge gaps while simultaneously aiding the realization of current operations was identified as valuable by several producers and supporting organizations. We have and will continue to communicate research results directly to stakeholders and producers through extension and outreach activities. A more thorough understanding of ʻiliahi biology and ecology is contributing to restoration, reforestation, and sustainable agriculture, while also generating environmental and social benefits by encouraging native ecosystem services and products for local communities.

Both field projects were located within ungulate-free fenced areas. Fencing is an essential first step to forest restoration and reforestation in Hawaiʻi because non-native ungulates inhibit natural regeneration, establishment, and growth. Maintaining fencing is critical to ensure native plant protection and for the possibility of natural regeneration and recruitment of diverse seedlings through developmental stages. In the host suitability project, our research provided evidence that ʻiliahi performs better when paired with koa as a host than with ʻaʻaliʻi as a host. Further, that closer planting distances support greater early growth. However, we recommend an intermediate distance that still provides enhanced growth and survival, but possibly has less potential for the trees to become entwined with each other.

The underplanting project provided evidence that ʻiliahi can survive and have exceptional early growth under the canopy of an established host. To compare the underplanting results to a control (i.e., ʻiliahi not underplanted), we tentatively drew comparisons to the host suitability project results where ʻiliahi seedlings of a similar size were planted simultaneously with a koa seedling. Average survival, height, and diameter were higher for underplanted ʻiliahi seedlings than for ʻiliahi seedlings planted simultaneously with koa seedlings as hosts. Since dry forests have been largely removed from the landscape, many restoration and reforestation efforts plant a mixture of species concurrently. Our results suggest that establishing hosts first may be worthwhile to increase the probability of more ʻiliahi establishing quickly. Since increased canopy openness increased ʻiliahi growth and physiology measurements, we recommend planting hosts at a lower density or thinning hosts to increase canopy openness before underplanting ʻiliahi.

From the 3-pot experiment it is confirmed that nitrogen is an important resource that ʻiliahi acquires from its host. Nitrogen tends to be the most limiting nutrient for plants; therefore, having a nitrogen-fixing host could help ʻiliahi to establish. This supports our finding from the host suitability project that koa, as a nitrogen-fixing tree, was a better host than ʻaʻaliʻi, a non-nitrogen-fixing shrub. Foliar nutrient results from the host suitability project also support the importance of nitrogen given that ʻiliahi had greater foliar nitrogen when paired with koa at the closer planting distances than the 2.0 m distance. The transfer of nitrogen also supports the underplanting results since ʻiliahi was planted under a koa canopy, therefore, there was likely more nitrogen in the soil under the koa canopy than would be without ten years of nitrogen-fixing trees being on site. Interestingly, our results provided evidence that root exudate transfer also occurred, which has implications for using stable isotope tracers as a tool in hemiparasite experiments.

5.4 Future Directions

This dissertation research followed the host suitability project for three years and the underplanting project for two years. It will be important to continue to follow these projects over time to assess how seedlings/saplings progress into the adult cohorts. The host suitability project will need to shift from an experiment with treatments to a neighborhood analysis because, already entering the fourth year after planting, koa's fast growth makes it likely that koa roots are accessible to ʻiliahi in control and ʻaʻaliʻi treatments. In the underplanting site additional species could be introduced to further diversify the reforestation stand and support restoration efforts. To support the 3-pot project, it would be instrumental to determine if haustoria formation can be encouraged to reduce how long the 2-pot and 3-pot units need to grow. Applying environmental stressors and evaluating if stress affects resource transfer would also be interesting and informative.

Two ongoing projects not part of this dissertation but pursue future research directions and support the presented dissertation research are (1) thinning koa and then underplanting ʻiliahi and (2) planting four ʻiliahi stocktypes in two substrates. The thinning and then underplanting project addresses whether identified crop koa can be enhanced by crown thinning while simultaneously creating more suitable conditions to underplant ʻiliahi. The stocktype project addresses whether certain stocktypes benefit early establishment and whether certain stocktypes perform better on certain substrate.

A few difficulties during the research process led to more questions. The most considerable difficulty was haustoria taking longer than expected to develop. During an initial trial of the 3-pot experiment, haustoria developed within six months. Under similar circumstances, during the experiment, there were no haustoria or very few haustoria after six months. Not only was this frustrating for experimentation, but it is concerning regarding ʻiliahi seedling quality as haustoria connections likely affect post-planting success. Are there certain nursery practices that increase haustoria formation? Could a treatment be applied (e.g., hormone) that facilitates haustoria development? Another difficulty was taking in situ leaf impressions. I tried superglue, gorilla glue, and clear nail polish to create impressions on microscope slides; both were difficult to create clean impressions. The difficulty could be a species-specific difficulty as impressions with other plants were not as difficult or due to the climate of the field site. Is there another method that may work better for taking ʻiliahi leaf impressions? Or would it be better to collect the leaves and sample in the lab rather than the field?

There are several additional avenues for ʻiliahi regeneration research including:

- Identifying a suite of hosts and their associated characteristics further to contribute to restoration efforts by encouraging diverse and robust restoration plans.
    - Does māmane, as another native nitrogen-fixing tree, support ʻiliahi as well as koa does?
    - What other native plants support ʻiliahi regeneration?
    - For sites where there are already non-native plants established (e.g., moringa, leucaena, casuarina, eucalyptus), can ʻiliahi be supported by them as hosts?
    - Could ʻiliahi suppress the growth and spread of invasive species (e.g., gorse)?
- Increasing heartwood and oil production.
    - Do certain hosts support more heartwood and oil production?
    - Does stress encourage heartwood and oil production?
- Other methods of detecting two-way transfer of resources.
    - Is ʻiliahi oil/other products consistently found it its host plants?
- Seed production, seed collection, seed zones.
    - Can seed production be encouraged for mature ʻiliahi?
    - Are there other methods to protect ʻiliahi seed from predation?
    - Should moving ʻiliahi seed be restricted to certain seed zones?
- What is the role of mycorrhizal fungi in hemiparasitic relationship?
- Can planted ʻiliahi with stagnant growth be supported to grow and develop into the sapling stage?
- Are nucleation plantings effective to create islands of regenerating ʻiliahi and hosts?
- ʻIliahi stress tolerance.
    - Drought
    - Frost
    - Light (both reduced light and shading effects by plants)

In future experiments, destructive sampling may be beneficial to measure root-to-shoot ratio and total tree biomass. These measurements could provide a more detailed comparison of resource allocation, plant moisture stress, and growth patterns. Comparing the costs of management treatments and potential effects on seedling growth would help land managers make informed decisions. A formal cost analysis of the various management options would help make decisions to restore dry forests and regenerate ʻiliahi. In conclusion, we presented research findings that support regenerating ʻiliahi in the context of Hawaiian dry.

As a valuable endemic tree, ʻiliahi can support ecosystem health and maintain critical ecosystem functions and services. ʻIliahi also has the potential to diversify local economies and support local communities because of its high value. There is great potential for restoration and reforestation, but for sustainable harvest to happen, appropriate efforts must be implemented. Sustainable ʻiliahi, in addition to koa, forestry could meet a balance between forest restoration and economic benefit. Eventually, a sustainable ʻiliahi harvest that follows appropriate forestry, cultural, and legal efforts in harmony with conserving designated areas can support restoration, reforestation, and conservation of dry forests.