

PLANT-PLANT INTERACTION IN EARLY- VS. LATE-SUCCESSIONAL VARIETIES OF  
THE HAWAIIAN LANDSCAPE-DOMINANT TREE, *METROSIDEROS POLYMORPHA*

By

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## Abstract

Plant-plant interactions play an important role in assembling plant communities. Interactions between neighboring plants can vary as a result of the genetic relatedness of neighbors, impacting rates of growth and patterns of resource allocation. When growing alongside close relatives, some species decrease their growth in a form of cooperation, while others grow faster through facilitation. A complication of plant interaction studies arises because decreased growth in the presence of close relatives can also be due to competition for resources, which increases with phenotypic similarity. Further complicating matters, mycorrhizal fungi, through their connections with plant roots, may strongly influence plant interactions.

My research compares the nature of plant-plant interactions within and between early- and late-successional varieties of Hawaiian trees, *Metrosideros polymorpha* var. *incana* (hereafter *incana*) and var. *glaberrima* (hereafter *glaberrima*), that differ in population density and the prevalence of mycorrhizal fungi in their native environments. I predicted that seedlings would respond (grow) differentially in the presence of genetically different neighbors and that the pattern of response would differ between the varieties in the presence/absence of mycorrhizae. Germinants of the two varieties were planted in pairs in experimental pots such that each pot contained a target seedling and a single neighbor, with the genetic relatedness of neighboring seedlings varying among treatments. One-half of the pots were supplemented with mycorrhizal fungi, and all were kept under ambient greenhouse conditions. After ~15 months, growth rates of the target seedlings were measured under the three treatments (sib = sibling neighbor, pop = neighbor derives from a different population of the same variety, and var = neighbor is from the opposite variety) as well as from control seedlings grown alone, both with and without mycorrhizal fungi. Additionally, hyphal growth was quantified in each pot with mycorrhizae to assess the

relative roles of cooperation versus competition; cooperating seedlings were expected to increase the flow of photosynthates (i.e., carbohydrates) to their mycorrhizal symbionts, thus increasing hyphal growth.

Based on the results, an increase in nutrient uptake with mycorrhizal fungi seems to be common in both the early- and late-successional varieties, but the seedling behaviors especially towards siblings seem to be different between *incana* and *glaberrima*. The overall growth of both varieties of *M. polymorpha* was increased, and allocation to root length relative to shoot length was reduced in the presence of mycorrhizae compared to the absence of mycorrhizae, suggesting an increase in nutrient uptake with mycorrhizal fungi. Differences among treatments were completely restricted to the sibling treatment versus the pop and var treatments. In the presence of sibling neighbors, target seedlings of *incana* and *glaberrima* had lower and higher root:shoot length ratios, respectively, than those grown with more genetically distant neighbors. Interestingly, root:shoot mass ratios did not vary among treatments for either variety. These results imply that seedlings of *glaberrima* grown with sibling neighbors prioritized vertical root growth over horizontal root growth to obtain nutrients. Also in the sibling treatment, but in the absence of mycorrhizal fungi, higher specific leaf area (SLA) of target seedlings was observed in both *incana* and *glaberrima*, but statistically supported only in *glaberrima*. In *glaberrima*, both the greater SLA of target seedlings grown with sibling neighbors and the constant shoot length among treatments suggest the ability of seedlings of this variety to increase light capture without shading sibling neighbors. Further, with a single exception, the sizes of target and neighboring seedlings were negatively correlated as expected, due to competition; the exception was the sibling treatment for which there was a significant positive relationship for *glaberrima* alone, not *incana*. Finally, the greatest mycorrhizal hyphal length was observed in both varieties in the sibling treatment,

suggesting cooperative behavior, yet this increase was statistically significant only in glaberrima. These four lines of evidence suggest that seedlings of late-successional glaberrima may be adapted to recognize and/or respond to genetically different neighbors, while such evidence was minimal or absent in seedlings of early-successional incana. Given that the differential response of seedlings to genetically different neighbors occurred even in the absence of mycorrhizal fungi, mycorrhizal symbionts do not appear to facilitate kin recognition or response in *Metrosideros*.

Beyond their responses to neighbor genetic relatedness, incana and glaberrima seemingly have different strategies for resource allocation affecting growth of both seedlings and their mycorrhizal symbionts that is consistent with their differential adaptation to early- and late-successional environments. Higher overall growth rates (i.e., final sizes) were observed in seedlings of incana relative to glaberrima regardless of neighbor treatment or the presence or absence of mycorrhizal symbionts. Rapid seedling growth in incana may reflect adaptation of this variety to the harsh conditions of early-successional environments where seedling establishment is likely restricted to occasional, brief periods of favorable conditions. In addition, mycorrhizal hyphal density was higher in incana-target pots relative to glaberrima-target pots. Despite a lack of correlation between total dry mass and hyphal density for incana or glaberrima separately or combined, the relatively higher growth rates of both target seedlings and mycorrhizal fungi in the incana pots may suggest a mutually positive relationship between incana and mycorrhizal fungi. Such a relationship may be expected in incana given the mycorrhizae-limiting nature and low population density characteristic of early-successional environments in Hawaii, where rapid seedling growth may be favored and can occur without intensifying competition among well-spaced neighbors. In contrast, growth of seedlings of glaberrima and their mycorrhizal symbionts may be restricted to minimize competition for resources in the high population densities of late-

successional forests. Results of this research provide novel insights into the poorly known world of neighboring interactions in trees affected by genetic relatedness and successional stage, with implications for forest restoration.

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## List of Abbreviations

SLA = specific leaf area

ECM = ectomycorrhizal

AM = arbuscular mycorrhizal

Pi = inorganic phosphate

N = nitrogen

P = phosphorus

SYM – symbiosis

PPA = prepenetration apparatus

ABA = abscisic acid

TDM = total dry mass

GLM = generalized linear model

Myco = presence or absence of mycorrhizal fungi as an independent variable

Trtmt = treatments (alone, sib, pop, var) as an independent variable

Var = varieties (var. *incana* and var. *glaberrima*) as an independent variable

Nhbr size = the size of the neighboring seedling as an independent variable

## **1. Plant-plant interactions**

### **1.1 Kin-recognition and cooperation in plants**

Plant-plant interactions play an important role in structuring plant communities. The genetic relatedness of neighboring plants can influence the nature of these interactions, which can vary between cooperation and competition (Cheplick & Kane, 2004; Dudley & File, 2007). In general, a strong competitor depletes resources from neighboring plants and suppresses their growth and/or reproduction, while a facilitative plant promotes the growth of neighbors (Armas et al., 2004). The fitness consequences of interactions among neighboring plants can vary as functions of both their genetic relatedness and ecological factors such as nutrient availability and dispersibility (Ehlers & Bilde, 2019). In addition to direct interactions between neighboring plants, mycorrhizal fungi, which have a mutualistic relationship with most plant species, may serve as a conduit for the exchange of substances and signals between neighbors (Marcelis & Bouwmeester, 2015). The nature of plant-plant interactions, therefore, may be expected to be altered in the presence of mycelial networks (Song et al., 2010).

Plants may show a plastic response in the presence of a neighbor (Bruce E. Mahall & Callaway, 1991) and to neighboring genetic relatedness by changing their growth (Dudley & File, 2007). Differential growth in response to the relatedness of neighboring plants requires kin recognition. Kin recognition, which is the ability to distinguish kin from non-kin, affects social behavior (Penn & Frommen, 2010). Kin are recognized when the spatial and temporal distributions of individuals overlap, and when individuals receive cues from the traits that other individuals express (Waldman, 1988). Kin recognition in plants occurs possibly through chemical exudates from roots, volatile molecules, electrical signals, and enzymes (Badri et al., 2012; Biedrzycki et al., 2010; Callaway & Mahall, 2007; Crepy & Casal, 2015; R. Karban et al.,



2013). Although the signals from other plants can be received by a plant both aboveground (R. Karban et al., 2013) and belowground (Biedrzycki et al., 2010), the importance of root interactions or soil properties (possibly containing root exudates) to recognize neighboring plants is emphasized in some studies (Burns & Strauss, 2011; Callaway & Mahall, 2007; Murphy & Dudley, 2009). The ability to recognize genetically different plants may help plants to determine cooperative or competitive behavior, to adjust territories, and to promote the success of relatives to enhance indirect fitness (Callaway & Mahall, 2007). Plants may allocate resources differently depending on the genetic relatedness of neighbors, and kin recognition therefore promotes fitness (Chen et al., 2012).

An individual can indirectly benefit through enhanced fitness of its relatives (kin), since some genes are shared among close relatives (Hamilton, 1964). In plants, traits favored by kin selection, such as reduced aboveground or belowground growth, may be costly to the actor since such traits would limit access to resources in the actor in return for avoiding competition with kin. As a result, the actor's own fitness (direct fitness) would be reduced while kin's fitness is increased. The cost of altruistic behavior could be compensated by increasing the chance to pass the shared genes to the next generations, thereby by increasing actor's relative's fitness (indirect fitness). This idea is mathematically formulated as Hamilton's rule,  $B \times r > C$ ; where B is the benefit the actor's kin can receive by actor's altruistic behavior, r is the relatedness coefficient, and C is the cost of the altruist actor (Hamilton, 1964). Altruism is a form of cooperation at the expense of an individual's own fitness, and kin selection favors altruistic behavior towards kin when its benefits exceed its costs. Altruistic behavior in plants may be influenced further by resource availability. For example, plants growing under low soil-nutrient and water availability in the presence of relatives may show cooperative behavior through reduced root growth (Dudley

& File, 2007), while plants growing among kin under low light may reduce leaf production to avoid shading of their closely related neighbors (Murphy & Dudley, 2009).

In direct contrast with the theory of kin selection, which predicts cooperation between closely related neighbors, niche theory (or resource partitioning theory) predicts that competition between neighboring plants should increase with their degree of genetic relatedness (Cheplick & Kane, 2004). Genetically distant individuals will have more phenotypic variation with greater size inequality than close relatives (Bhatt et al., 2011). Niches are expected to be less overlapped between strangers than between relatives, and this leads to lower competition and higher fitness between co-occurring strangers. On the other hand, the phenotypic similarity of close relatives, and thus their similar strategies for nutrient uptake, are expected to result in more intense competition between neighboring plants. According to niche partitioning theory, the greater strength of intraspecific competition relative to interspecific competition promotes the coexistence of disparate species (or phenotypes) (Chesson, 2000). The complication arises in studies of plant behavior in response to neighboring genetic relatedness, because when growing in the presence of close relatives, some plant species decrease growth through cooperation (Dudley & File, 2007) and others through competition (Cheplick & Kane, 2004). Because both positive and negative consequences of neighboring-plant interactions can manifest as reduced growth (Ehlers & Bilde, 2019), it is challenging to understand the nature of plant-plant interactions based on observations of plant growth alone.

Because of the difficulty of deciphering the nature of plant-plant interactions, the importance of kin selection in plants is still controversial (Ehlers & Bilde, 2019). Evidence of kin selection derives primarily from the plastic response of plants to closely related vs. distantly related neighbors, usually shown as changes in plant growth rather than direct measurements of

fitness (Dudley & File, 2007). Given that the reduced growth with close relatives could be due to cooperation or competition, plant growth alone does not provide us with conclusive evidence that the traits that share the limiting resources increase fitness with kin (Ehlers & Bilde, 2019). Furthermore, there are no standardized traits to indicate altruistic behavior in plants, and traits shown to be cooperative may be specific to the species studied or their natural environments (Dudley & File, 2007; Murphy & Dudley, 2009). Therefore, to show evidence of kin selection, it is necessary to demonstrate an increase in the fitness of relatives resulting from the cooperative behavior (= inclusive fitness) along with plasticity of traits in response to genetically different neighbors (Ehlers & Bilde, 2019).

The fitness consequences of neighbor interactions may also be influenced by the frequency with which those interactions occur in nature. With a high frequency of interactions between kin, kin selection favors cooperative behavior, resulting in the expansion of kin populations within the community and positive frequency-dependent interactions that promote the coexistence of close relatives (Ehlers & Bilde, 2019). This is in direct contrast with the negative frequency-dependent interactions within ecological communities that are expected to promote the coexistence of diverse species or phenotypes (Kimura & Ota, 1971). A good example of positive frequency-dependent interactions among kin can be seen in the white clover, *Trifolium repens*, in which competitive and reproductive traits were compared between plants grown in the experimental pots under low and high population densities of siblings and non-siblings (Lepik et al., 2012). Because the field community comprised ~45% *T. repens*, the frequency of interactions among kin was expected to be high. Plants of *T. repens* demonstrated no significant change in petiole length between low and high density, but increased specific leaf area (SLA), and increased inflorescence mass with high density of siblings (Lepik et al., 2012).

The authors concluded that kin selection favored traits that reduced competition while increasing light-capture efficiency and reproduction in the presence of a high density of siblings.

Cooperative behavior in this species may though allow kin to coexist even at high density through positive frequency-dependent interactions.

## **1.2 Competitive ability in plants**

When two plants compete, one or both will deplete resources from the other(s), and the plant experiencing resource depletion will respond through suppressed growth or increased efficiency of resource uptake. How focal plants affect their neighbors is depicted as the competitive effect, which is defined as a plant's ability to suppress neighbor growth (D. E. Goldberg, 1996; Deborah E. Goldberg, 1990). The response of the focal plants to the neighboring competitor on the other hand is known as the competitive response. Competitive response is defined as the ability of a plant to survive and grow in the presence of a competitor. Since resource depletion is the primary means by which plants compete, above- and belowground competitive effects vary with the depletion of resources by the focal plant on the competitor. Thus, the competitive effect is often measured as the reduction in competitor's fitness with and without the focal plant (Dudley et al., 2013). In contrast, the measurement of the competitive response is focal plant's fitness in the presence of the competitor.

In plants, the competitive effect on neighbors is basically negative through growth suppression, while competitive response traits appear as traits that increase fitness of plants in the presence of competitors. Plants with strong competitive effects, such as many invasive species, are strong competitors in many plant communities (Thomsen et al., 2006). Plants having a strong competitive effect can deplete more resources from the shared zone with the

neighboring competitor and enhance their own growth while the neighbor will probably struggle with the limited resource to grow and survive. The competitive effect will increase growth and survival of the focal plants by reducing the neighbor's growth. The competitive response, on the other hand, is a passive behavior in plants and depends on the ability of plants to grow and survive within the limiting resources in the presence of competitors. The competitive response is directly linked to the target plant's fitness under competitive conditions. Since natural selection always favors traits that increase fitness (Orr, 2009), competitive-response traits that maximize fitness are favored by selection (Dudley et al., 2013), especially when multiple plants interact (Aschehoug et al., 2016). Thus, competitive-response traits are plastic traits that are favored in the presence of competitors but not in their absence (Dudley et al., 2013).

Vertical growth in plants can directly increase resource acquisition. The higher plants grow aboveground, the more they can intercept light, whereas the deeper plants grow underground, the more they can absorb soil nutrients. In a competitive environment, the competitive effect on neighboring plants may appear as reduced neighbor height and root depth under low light and water availability, respectively (Violle et al., 2009). A strong competitor will deplete resources and increase growth while limiting access to resources by the neighbor. Thus, resource depletion through both above- and belowground competition may be directly linked to competitive-effect traits, including the vertical growth of neighboring plants. Competitive response traits, in contrast, may appear in the form of horizontal growth or physiological changes (Violle et al., 2009). For example, specific leaf area ( $SLA = \text{leaf area} / \text{leaf mass}$ ) tends to increase as light availability decreases in general (Poorter & Nagel, 2000). SLA is a competitive response trait that alters light absorption per unit leaf mass under low light and is a good predictor of plant performance in the presence of a competitor (Violle et al., 2009). Additionally,

since leaf water potential is a function of water availability (Boyer, 1995), leaf water potential and also root radius (Jastrow & Miller, 1993) may also serve as competitive response traits.

Competition is strongest when soil nutrients, light, and space are limited. Under low resource availability, both competitive-effect traits and competitive-response traits may be needed (P. Wang et al., 2010). Nonetheless, competitive effect traits are not necessarily correlated with competitive response traits, especially when the resources and spaces are unlimited (P. Wang et al., 2010). Competitive-effect and -response traits may occur simultaneously within a single individual (Violle et al., 2009), and a single trait may manifest as either a competitive effect or response (Dudley et al., 2013). For example, petiole length can respond to light availability, elongating to enhance light absorption (H. Smith, 1995). Petiole elongation may result in the shading of neighbors as a competitive effect, and at same time, in the avoidance of self-shading as competitive response (Dudley et al., 2013). This mixed effect leads to difficulty in distinguishing between competitive-effect and -response traits.

Since competitive interactions between neighboring plants may be affected by whether the neighboring plant is closely or distantly related to the focal plant, the competitive ability, especially the competitive response, would be an important measurement to understand how kin selection acts on plants (Dudley et al., 2013). The effect of phenotype of the focal plant on relative's fitness would be equivalent to competitive effect, which is generally negative to less negative depending on genetic relatedness (Dudley et al., 2013). The negative competitive effect could be interpreted as selfish behavior, expected with strangers under kin selection theory. If the competitive effect is less negative, the focal plant is less competitive to the neighbor, indicating avoidance of competition and ultimately cooperation, more likely with siblings. To study kin selection, therefore, we need to know inclusive fitness in the plant community,

including how the focal plant might affect the neighboring plant (competitive effect) and how the focal plant might respond to the neighboring plant (competitive response). Unlike the competitive effect which mainly has a negative impact on the neighbor, the competitive response is considered to be a cooperative behavior. Measurement of the competitive response would be required to understand the nature of interactions between closely and distantly related neighboring plants (Dudley et al., 2013).

### **1.3 Plant-plant interaction between self vs. non-self**

As is true for animals, plants can communicate with each other. Communication entails sharing information between the sender and the receiver (Simard, 2018), and interplant signaling plays a role in communication (Gagliano, 2013). The interaction between plants can affect plant growth and survival, and ultimately shape the plant community. Research on plant-plant communication started relatively recently, and pioneering work highlighted the importance of airborne signals between neighbors (Baldwin & Schultz, 1983; Rhoades, 1983). In a separate study, growth of roots away from non-self was observed even without physical contact suggesting that plants can use soilborne signals to recognize neighboring plants (Bruce E. Mahall & Callaway, 1991). Plants, it seems, have the ability to recognize and respond to neighboring plants through both above- and below-ground cues. More recently, the importance of mycorrhizal networks as conduits between neighboring plants is being recognized (e.g., Song et al., 2010). A majority of studies have found that belowground plant-plant interaction plays an essential role in determining plant growth and survival through roots, root exudates, and mycorrhizal fungi (Simard, 2018).

The ability to distinguish between self and non-self may be essential for organisms to survive and grow. The immune system is a good example at the cellular level of discrimination of ‘self cells’ from ‘non-self cells’ (Richard Karban & Shiojiri, 2010). Sixty percent of angiosperms show some form of self-incompatibility (Sanabria et al., 2008), indicating that a majority of angiosperms choose mates based on whether the mate is self or non-self (Depuydt, 2014) through the control of pollen germination or pollen tube growth, for example (Richard Karban & Shiojiri, 2010). The first evidence of a plant’s ability to distinguish between self and non-self was documented in the desert shrub (*Ambrosia dumosa*), which showed a reduced rate of root elongation when contacting non-self roots but no effect of self roots on root elongation rate (Mahall & Callaway, 1991). This study suggested that plant roots tend to avoid contact with other roots from the same plant. Although the ability to distinguish self from non-self could allow the avoidance of intra-plant competition, self/non-self recognition does not always link to the avoidance of self- roots since the underlying mechanisms may be species specific (Depuydt, 2014). Some plants showed no difference in root growth in the presence of self versus non-self roots (Markham & Halwas, 2011), while others actually increased root growth in the presence of self-roots (Gruntman & Novoplansky, 2004).

Plants can recognize self/non-self both below- and aboveground, yet physical contact seems to be necessary for self-recognition belowground. Physical contact between roots seems to be more important than root exudates for distinguishing self from non-self (Caffaro et al., 2011; Richard Karban & Shiojiri, 2010). Given that self and non-self exudates similarly suppress root growth, root exudates alone are not likely to influence self/non-self recognition (Caffaro et al., 2011). Rather, roots are likely to recognize self only when they are in physical contact (Depuydt, 2014). In addition to self-recognition below-ground, some plants are able to



recognize self above-ground (Richard Karban & Shiojiri, 2009). For example, sagebrush plants can induce herbivore resistance more efficiently in response to the volatile signals emitted by self clones compared to those from non-self clones. In sum, physical contact appears to be necessary to distinguish between self and non-self belowground but not necessary aboveground.

#### **1.4 Impact of neighboring genetic relatedness on plant behavior**

In addition to self/non-self recognition, how plants interact with genetically varying individuals can have a significant impact on their fitness. Plant growth and survival can be affected by neighbors of different genotypes, families (siblings vs. non-siblings), populations, and species (Andalo et al., 2001; Lee et al., 2020; Bruce E. Mahall & Callaway, 1996; A. L. Smith et al., 2019). Relative to plant-plant interactions aboveground, interactions between roots appear to be more important at the initial stages of interaction between genetically different neighbors (Murphy & Dudley, 2009). In contrast to self-recognition belowground that requires physical root contact, kin recognition can be effected by root exudates without physical interaction (Biedrzycki et al., 2010). Therefore, self-recognition and kin recognition may occur through different mechanisms (Depuydt, 2014).

When a plant's neighbor is a relative, such as a sibling, the expected outcome is either intense competition based on the niche partitioning theory or cooperation according to kin selection theory. Niche partitioning theory is supported in studies that reveal relatively higher fitness in plants grown with distantly neighbors relative to those grown with closely related neighbors (Cheplick & Kane, 2004). In studies of sibling neighbors, however, cooperative behavior is more often observed, rather than intense competition (Biedrzycki et al., 2010; Crepy & Casal, 2015; Murphy & Dudley, 2009). As a partial reason why plant response varies toward

siblings, some researchers have pointed out problems of study designs that involve the comparison of groups, such as a sib group and a non-sib group, or the performance of a target plant grown alongside a sib group or a non-sib group (Bhatt et al., 2011; A. L. Smith et al., 2019). Instead of examining plant behavior in a group setting, measuring the competitive ability of paired plants would be a better research design to understand plant interactions with close relatives (Bhatt et al., 2011). Since mutualistic mycorrhizal fungi also play an important role in kin recognition (Ronsheim & Anderson, 2001), the presence of mycorrhizal symbionts should also be taken into consideration in such studies.

Cooperative behavior towards siblings is likely related to the population structure characteristic of a plant species. Cooperative behavior is often, but not always (Milla et al., 2009), seen in dispersal-limited plants where interactions between genetically related individuals are frequent (i.e., a viscous population) (Murphy & Dudley, 2009; Platt & Bever, 2009). Limited dispersal usually increases genetic similarities and interactions with close relatives in a population (Platt & Bever, 2009). Thus, population viscosity tends to promote the evolution of cooperative traits.

Cooperation usually appears in the form of reduced growth in the presence of related individuals (i.e., the avoidance of competition) both above and below ground (Biedrzycki et al., 2010; Cheplick & Kane, 2004; Dudley & File, 2007). Yet sometimes the interpretation of plant behavior is not so simple. In *Impatiens pallida* which usually compete for light, an increase in leaf-to-root allocation with strangers was interpreted as above-ground competition with strangers while an increase in elongation and branchiness with sibs was interpreted as decrease in interference with kin (Murphy & Dudley, 2009). Overall, results of the study of *I. pallida*

suggested that siblings cooperated with each other by avoiding competition aboveground (Murphy & Dudley, 2009).

Total or aboveground biomasses are often used as the main response variable to understand plant-plant interaction based on the idea that biomass is strongly linked to reproduction and fitness (Aschehoug et al., 2016). However, it is sometime risky to rely on one measurement since biomass does not always show plant behavior clearly (File, Klironomos, et al., 2012). Furthermore, an increase in one part of a plant (e.g., increased elongation) should not be simply interpreted as a competitive response (Murphy & Dudley, 2009). A study on plant-plant interactions with multiple measurements including the length of root and shoot, for example, in addition to biomass is needed to provide conclusive evidence of plant behaviors towards siblings.

Shifts in a plant's competitive ability depending on intra- and interspecific interactions can reveal the important of short- versus long-term fitness (Padilla et al., 2013). In intraspecific interactions, *Festuca rubra* is a stronger competitor than *Plantago lanceolata* by having high root density (Padilla et al., 2013), as a competitive advantage to deplete resources (Aschehoug et al., 2016). In interspecific interactions, however, *F. rubra* became a weaker competitor by reducing root and shoot growth with *P. lanceolata*, while *P. lanceolata* increased growth with *F. rubra*. Increasing root density is beneficial in *P. lanceolata* in the presence of *F. rubra* as a short-term result. In fact, *P. lanceolata* dominated the area first. Over time, *F. rubra* became dominant, suggesting that the short-term positive result does not necessarily result in the same positive result over a long period. According to two studies (Lee et al., 2020; Padilla et al., 2013), understanding both intra- and interspecific interactions is necessary to understand the big picture of plant-plant interactions.

In ecological communities, early- and late-successional plant species have different strategies, including growth rate, decomposition rate, and responsiveness to mycorrhizae (Koziol et al., 2015; Rothstein et al., 2004; Seifert et al., 2009). Fast growth is typical of early-successional species, while slow growth is often seen in late-successional species (Koziol et al., 2015). In the most productive environments, competitive (i.e., fast-growing) plant species can dominate the vegetation, while slow-growing plants should be favored in less productive habitats to prevent resource depletion (Grime, 1977). Plants that have different strategies may affect competitive outcomes when early- and late-successional forms interact. When seedlings of late-successional *Paraberlinia bifoliolate* were grown under early- or late-successional adult trees, the lowest survival and ectomycorrhizal formation was found under the early-successional tree species (Onguene & Kuyper, 2002). The highest survival rate was observed when *P. bifoliolate* was grown under another late-successional tree species, not the same species, highlighting the conspecific interaction did not result in the highest survival rate. This reason could be explained by the Janzen-Connell model, which predicts high mortality in conspecific interactions due to host-specific predation or parasitism (Connell, 1971; Janzen, 1970). Importantly, in addition to impacts on plant growth and survival from intra- and interspecific interactions, plant interactions may also be strongly influenced by successional stage.

Plant-plant interactions affect plant behavior changing plant density, and ultimately shaping community structure. At the species level, conspecific interactions may result in negative feedback by reducing survival (Onguene & Kuyper, 2002). This phenomenon is known as conspecific negative density dependence (Lebrija-Trejos et al., 2016). Conspecific negative density dependence can promote coexistence by limiting individual species (Chesson, 2000).

In summary, plant-plant interactions can occur both above- and belowground, and plants have the ability to distinguish between self and non-self as well as to recognize neighboring genetic relatedness affecting their growth and survival (Fig. 1.1). Although more study is needed on the mechanisms of plant interactions, plants are likely to have different mechanisms for self-recognition and kin recognition. In addition to the effect of neighboring plant types on plant growth and survival, different characteristics in plants associated with ecological functions such as the successional stage likely also play an important role in influencing plant-plant interactions.

Relationship	Parameter	Above- and/or belowground interactions	Effect of neighbors	Species	Reference
Self and non-self	Root elongation rate	Belowground	Reduced with non-self roots, no effect with self roots	<i>Ambrosia dumosa</i>	Mahall & Callaway 1991
	Root length and/or number	Belowground	Reduced with both self and non-self roots	<i>Larrea tridentata</i>	Mahall & Callaway 1991
		Belowground	No effect	<i>Andropogon gerardii</i>	Markham & Halwas 2011
Same and mixed genotypes	Root length and/or number	Belowground	Increased root length and number with self-roots	<i>Buchloe dactyloides</i>	Gruntman & Novoplansky 2004
		Belowground	No effect of self/non-self exudates	<i>Arabidopsis thaliana</i>	Biedrzycki et al. 2010
	Resistance to herbivory	Aboveground	Herbivore resistance induced in response to the volatile signals by self-clones	<i>Artemisia tridentata</i>	Karban & Shiojiri 2009
Siblings and non-siblings	Fruit production	Both	Increased with same genotypes with ambient CO2 level	<i>Arabidopsis thaliana</i>	Andalo et al. 2001
	Leaf orientation	Both	Reoriented leaf away from kin	<i>Arabidopsis thaliana</i>	Crepy & Casal 2015
Siblings and non-siblings	Above- and/or below ground growth	Both	Reduced biomass with siblings relative to nonsiblings	<i>Aegilops triuncialis</i>	Smith et al. 2019
		Both (importance of root interaction is highlighted)	Increased branchness with sibs (= decreased interference with kin)	<i>Impatiens pallida</i>	Murphy & Dudley 2009
Intra- and interfamily	Root length and/or number	Belowground	Greater number of lateral root with non-sibling exudates than sibling exudates	<i>Arabidopsis thaliana</i>	Biedrzycki et al. 2010
	Shoot and seed mass	Both	Increased with distantly related family	<i>Triplasis purpurea</i>	Cheplick & Kane 2004
Same and different population	Root elongation rate	Belowground	Reduced with roots from the same population, no effect of roots from the different population	<i>Ambrosia dumosa</i>	Mahall & Callaway 1996
Intraspecific	Phenolic compounds	Aboveground	Increased in both damaged and undamaged plants that were in the same enclosure	<i>Populus euroamericana</i>	Baldwin & Schultz 1983
		Aboveground	Increased in both damaged and undamaged plants that were in the same enclosure	<i>Acer saccharum</i>	Baldwin & Schultz 1983
	Leaf quality	Aboveground	Altered in undamaged neighbors in response to signals released from damaged trees	<i>Alnus rubra</i>	Rhoades 1983
		Aboveground	Altered in undamaged neighbors in response to signals released from damaged trees	<i>Salix sitchensis</i> <i>Festuca rubra</i> & <i>Plantago lanceolata</i>	Rhoades 1983 Padilla et al. 2013
	Above- and/or below ground growth	Both	Higher root density in <i>F. rubra</i> than to <i>P. lanceolata</i>	<i>Festuca rubra</i> & <i>Plantago lanceolata</i>	Padilla et al. 2013
Interspecific	Above- and/or below ground growth	Both	Reduced root and shoot growth in <i>F. rubra</i> with <i>P. lanceolata</i>	<i>Festuca rubra</i> & <i>Plantago lanceolata</i>	Padilla et al. 2013
	Survival	Both	Invasive species outperformed native species	<i>Taraxacum officinale</i> & <i>T. platycarpum</i>	Lee et al. 2020
		Both	Lowest survival in late-successional <i>P. bifoliolate</i> with the early-successional species and highest survival with another late-successional species	<i>Paraberlinia bifoliolate</i> (late-successional) with early- and late-successional species	Onguene & Kuyper 2002

Fig. 1.1 Overview of plant-plant interactions.

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## **2. The effect of mycorrhizal fungi on plant-plant interactions**

### **2.1 Types of mycorrhizal fungi and benefits of plant-mycorrhizal mutualisms**

Almost all land plant species have mutualistic relationship with microbes such as mycorrhizal fungi and rhizobial bacteria. There are two major groups of mycorrhizal fungi, depending on the fungal structure and function: endomycorrhizal and ectomycorrhizal (ECM) fungi (Fig. 2.1). Endomycorrhizas can be further divided into arbuscular mycorrhizal (AM) fungi, ericoid, and orchid (Bonfante & Genre, 2010). Current endomycorrhizas belong to Glomeromycota, while ectomycorrhizas are in Ascomycota and Basidiomycota. A similarity between the two classes is that neither endo- nor ectomycorrhizas contact the cytoplasm of the host plant. A major difference is that endomycorrhizas colonize within newly formed plant plasma membranes inside the plant cell, whereas ectomycorrhizas colonize the intercellular spaces of the root (Bonfante & Genre, 2010). AM and plants have an obligate mutualistic relationship, meaning AM are not able to survive in the absence of the host plants (Bonfante & Genre, 2010). On the other hand, ECM can live independently as shown by the capacity to grow in petri dishes (S. E. Smith & Read, 2008). Thus, it appears that ECMs can occupy dual niches: in the soil as saprotrophs and in the host plant as symbionts (Bonfante & Genre, 2010).

Groups	Endomycorrhizas	Ectomycorrhizas
Classification	Arbuscular Ericoid Orchid	-
Phylum	Glomeromycota	Ascomycota Basidiomycota
Contact the cytoplasm of the host plant?	No	No
Colonization	Inside the plant cells	Around root tips Between root epidermal cells
Relationship with host plants	Obligate mutualistic with plants	Can survive without plants
The oldest evidence	Triassic (215 - 235 Ma)	Jurassic (190 Ma)
# of host plant species associated with	~200,000	~6,000
Main distribution	Lower latitudes	Intermediate altitudes and latitudes
Ecosystem	Species-rich	Forests with a few host species
Main ecological function	Mobilize phosphate	Mobilize nitrogen

Fig. 2.1 Overview of endo- and ectomycorrhizal fungi.

The history of the mutualistic association between plants and mycorrhizal fungi probably started upon the emergence of land plants, although evidence for this remains inconclusive. Colonization of land by early plants began during the Mid-Ordovician (~470 Ma) (Martin et al., 2017). The discovery of arbuscule-like structure (Glomeromycota) and intracellular coils (Mucoromycotina) in fossilized plants (407 Ma) suggests associations between lower land plants (i.e., plants lacking true roots) and a potentially more diverse AM community than occurs today, which comprises the Glomeromycota only (Remy et al., 1994; Strullu-Derrien et al., 2014). It is possible that terrestrialization of plants was facilitated by fungal associations (Bidartondo et al., 2011). In the Late Carboniferous period (~300 Ma), continents were covered by extensive swamp forests comprising tree-like plants with fine rootlets, arborecent lycopsides (distant relatives of present-day herbaceous mosses) forming mycorrhizal symbioses with AM-like fungi (Krings et al., 2011). The oldest evidence of AM fungal associations with gymnosperms (e.g., cycads and conifers) is documented from the Triassic period (215-235 Ma) (Phipps & Taylor, 1996). The earliest observed ECM association in Pinaceae species is in the Jurassic (190 Ma),

and ECM evolved many times independently since then (M. C. Brundrett, 2002; Lambers, Stuart Chapin, et al., 2008). Angiosperms started to dominate the landscape during the Cretaceous period, having both AM and ECM symbioses (Martin et al., 2017; Matheny et al., 2009).

Mycorrhizal fungi occur in most environments, including alpine zone, tropical, temperate, and boreal forests, grasslands, and croplands (Bonfante & Genre, 2010; Van Der Heijden et al., 2015). In general, AM fungi are associated with herbaceous and woody plant species at lower latitudes, including tropical forests and temperate grasslands, while ECM species are found in forest ecosystems at intermediate altitudes and latitudes ranging from the tropics to Eurasia/North America (Read, 1991). AM fungi are often found in species-rich ecosystems in mineral soils, whereas ECMs predominate in forest ecosystems where only a few host species are present in organic soils (Lambers, Stuart Chapin, et al., 2008; S. E. Smith & Read, 2008). There is typically a low abundance of mycorrhizal fungi in very young soils (Gemma & Koske, 1990; Lambers, Raven, et al., 2008). Studies of soil chronosequences reveal a change in nutrient availability from high total soil phosphate through a phosphate-nitrogen-limiting phase, and finally a nitrogen-limiting phase (Dickie et al., 2013). Because AM and ECM fungi mobilize phosphate and nitrogen, respectively, more efficiently, there may be a correlation between these shifts in soil nutrients and changes in the dominant mycorrhizal types along chronosequences (Lambers, Raven, et al., 2008), although this relationship remains controversial (Dickie et al., 2013).

Mutualistic relationships with mycorrhizas are found in 82% of all angiosperms and all gymnosperms (Lambers, Stuart Chapin, et al., 2008). AM fungi have established mutualistic relationships with ~200,000 plant species including poplars, eucalypts and some gymnosperms, whereas ECM have symbioses with ~6,000 species including pines, beeches, oaks, eucalypts,



dipterocarps and poplars (S. E. Smith & Read, 2008; Van Der Heijden et al., 2015). Some plants cluster roots to efficiently mobilize and absorb nutrients while establishing both arbuscular mycorrhizal and N<sub>2</sub>-fixing rhizobial symbioses; these include *Casuarina* (Casuarinaceae), *Alnus* (Betulaceae), *Myrica* (Myricaceae) and *Viminaria* (Fabaceae) (Lambers et al., 2003). It seems mycorrhizas are less important for some fast-growing plants (Brassicaceae and Chenopodiaceae), which are usually found on disturbed and nitrogen-limited soils where relationships with N<sub>2</sub>-fixing symbionts are favored (Lambers, Raven, et al., 2008).

The benefits for host plants of mutualistic relationships with mycorrhizal fungi are numerous. First, extensive mycelial development around plant roots increases surface area and distance to absorb and transport nutrients from soil. The longest distance of nutrient transport through a mycelium is reported as 25cm (Lambers, Raven, et al., 2008). The increase in nutrient-absorbing surface area is important especially for phosphate uptake because inorganic phosphate (Pi), which is the only form of phosphorus available to plants, moves very slowly and has low solubility in soil (Lambers, Raven, et al., 2008; Silberbush & Barber, 1983). Because root absorption of Pi is faster than Pi mobility in soil, the concentration of Pi around roots decreases quickly. The development of an extensive extraradical mycelium network can overcome the depletion of Pi close to roots (Lambers, Raven, et al., 2008).

Second, in addition to an increase in the nutrient absorption area, mycorrhizas facilitate acquisition of mineral nutrients for plants, even when the minerals are not in plant-available forms (Bonfante & Genre, 2010; Lambers, Stuart Chapin, et al., 2008). AM contribute significantly to the transport of Pi to plants, while ECM exploit insoluble organic forms through the exudation of enzymes (Li et al., 2006). This is supported at the molecular level by the presence of transporters of Pi in AM, and some organic nitrogen (N) forms as well as genes to

transfer the nutrients in ECM (Bonfante & Genre, 2010). Some mycorrhizal fungi may even have the ability to capture organic P and other N forms, which are not absorbed directly by plant roots, and transport these compounds to plants along with plant-available forms (Hodge et al., 2001; Lambers, Stuart Chapin, et al., 2008). Interestingly, once AM fungi colonize plants, Pi uptake through roots can be reduced and switched to mycorrhizal-indirect uptake (Li et al., 2006; Poulsen et al., 2005; S. E. Smith et al., 2004), suggesting a dependency of plants on a Pi supply from mycorrhizal fungi. A significant increase in plant growth is often documented with the addition of mycorrhizas under otherwise constant soil conditions (Gemma et al., 2002).

Third, biotic and abiotic stresses can be mitigated in the presence of mycorrhizal fungi. To protect against biotic stress, mycorrhizal mycelial networks connecting plant roots may send a signal to warn neighbors when a plant is attacked by herbivores (Babikova et al., 2013). Upon warning, the neighboring plants can increase their tolerance to herbivore attack by attracting predators or changing chemical components before physical damage by herbivores has occurred (Johnson & Gilbert, 2015). Resistance to abiotic stress, including tolerance to drought or high-salinity conditions as well as mineral depletion, is well reported in plants colonized by mycorrhizal fungi. For example, AM fungi can regulate the integrated physiological response of the host plant to improve salinity tolerance by reducing osmotic potential and maintaining water uptake (Ruiz-Lozano et al., 2012; Vicente-Sánchez et al., 2014). Furthermore, plant survival and growth can be increased even under soil moisture stress when the plants are connected through mycorrhizal networks that aid access to water resources (Bingham & Simard, 2011).

A well-known benefit to mycorrhizal fungi of the mutualistic relationship with plants is the reception of carbohydrate products of photosynthesis by the host plant. In ECM (*Laccaria bicolor*), 15 genes are annotated for putative hexose transporters (Bonfante & Genre, 2010).

Interestingly, almost zero genes for invertase, which hydrolyses sucrose (the most abundant form of sugar) to glucose and fructose, are found in ECM (0 gene in *L. bicolor*, 1 in *Tuber melanosporum*) (Bonfante & Genre, 2010). Given that pathogenic fungi have sucrose transporters that allow them to take in sugar without the aid of invertase (Wahl et al., 2010), the lack of the invertase enzyme in ECM is consistent with their dependency on a stable mutualistic relationship with the plant host as a carbon source. In AM fungi, a sugar transporter (*MST2*) was identified with the function to transport not only glucose but also other sugars derived from the plant cell wall (Helber et al., 2011). Since *MST2* expression is closely correlated with that of phosphate transporter (*PT4*), the exchange of carbon for phosphate should be tightly linked (Helber et al., 2011).

## **2.2 Hormonal regulation of the plant-mycorrhizal symbiosis**

The relationship between mycorrhizal fungi and host plants extends back over 400 My (Martin et al., 2017). Several lines of evidence indicate that both organisms accept each other as partners at the molecular and organ levels. Hormones play a crucial role in establishing symbiotic relationships between mycorrhizal fungi and plants, and the two partners typically use the same hormones, but with different intentions (Eichmann et al., 2021). Both plant-derived and microbial hormones can affect root architectures and plant defense against fungal species (Petricka et al., 2012; Vanstraelen & Benková, 2012), helping to stimulate and establish the symbiotic relationship (Eichmann et al., 2021). Although the mechanisms by which AM fungi establish symbioses with host plants is well characterized, understanding of how ECM and plants interact at the molecular level is limited.

The interaction between mycorrhizal symbionts and plant roots can begin even before they achieve physical contact, through root exudates and mycorrhizal hormones. Plants stimulate mycorrhizal symbionts by exuding the carotenoid-derived phytohormones, strigolactones (Landgraf et al., 2012). Plants use strigolactones to suppress shoot branching and to regulate root development, including primary root growth, lateral root formation, and root hair elongation (Koltai, 2015). Although the receptor for strigolactones has yet to be found in AM fungi (Eichmann et al., 2021), AM fungi respond to strigolactones in root exudates by producing diffusible signals, including lipochitooligosaccharides known as Myc factors (Maillet et al., 2011). Perception of strigolactones by AM fungi results in an increase in the  $\text{Ca}^{2+}$  concentration in the fungal cytoplasm along with spore germination and hyphal branching (Lanfranco et al., 2016; Waters et al., 2017).

When the plant senses Myc factor signals, a symbiosis (SYM) pathway involving at least seven genes (SYM genes) is reprogrammed to prepare the plant for establishment of symbiotic interactions with AM fungi and even with nitrogen-fixing rhizobia (Bonfante & Genre, 2010). Repeated oscillation of  $\text{Ca}^{2+}$  concentrations in the nuclear regions of root hairs (called calcium spiking) is another change caused by Myc factors (Bonfante & Genre, 2010). Once fungal hyphae contact the plant root, the hyphae modify their structure and form hyphopodia on the root surface, which triggers activation of several plant genes in the hyphopodium region (Bonfante & Genre, 2010). In response to fungal contact, plants produce an AM-specific structure under the hyphopodium called a prepenetration apparatus (PPA) (Bonfante & Genre, 2010). Calcium spiking stimulates the formation of the PPA (Gutjahr & Parniske, 2013) that allows fungal hyphae to penetrate through the plant root (Chabaud et al., 2011). Subsequently, fungal hyphae

grow intercellularly, and tree-like structures called arbuscules are formed to exchange nutrients and water between fungi and plants (W. Wang et al., 2017).

In addition to strigolactones, a properly balanced set of hormones control the symbiotic associations between host plants and mycorrhizal fungi, sometime synergically. At the stage of root colonization, a number of hormones that regulate colonization have been identified, including, but not limited to, abscisic acid (ABA), auxin such as indole acetic acid, cytokinin, and ethylene (Hilbert et al., 2012; Pozo et al., 2015; Stec et al., 2016). The function of ABA might be associated with strigolactones and arbuscule formation, given the correlated abundances of these hormones (Stec et al., 2016). Flavonoids, which are well-known plant stress hormones, also play an important role in the symbiosis by stimulating hyphal branching and mycorrhizal growth (Bais et al., 2006). A variety of hormones are therefore essential for the development of the symbiotic relationship between mycorrhizas and their host plants, from the recognition of compatible hosts and symbionts to the establishment of symbiosis.

In contrast to the established role of strigolactones in the development of AM-plant interactions (Lanfranco et al., 2016), the significance of strigolactones for ECM-plant symbioses is not documented (Garcia et al., 2015). Given evidence of no effect of strigolactones on ECM growth (Steinkellner et al., 2007), it is not clear if strigolactones have a positive influence on ECM symbiosis. Although Myc factors such as the chitoooligosacchides that are produced by AM fungi to stimulate symbiosis with plants are not found in ECM (Garcia et al., 2015), ECM may be still able to release chitoooligosacchides as a by-product of cell wall remodeling to colonize host plant roots (Adams, 2004). Several hormones are known to enhance ECM growth and/or stimulate spore germination, including flavonoids, auxin, and cytokinin (Debaud & Gay, 1987; Gogala, 1991; Lagrange et al., 2001). On the other hand, ethylene and jasmonic acid may

prevent fungal colonization (Garcia et al., 2015). By reducing the abundance of negative regulators such as jasmonic acid affecting the plant defense pathway, plants may allow ECM hyphal development in the root.

Plants defend against pathogenic fungi by producing root exudates and altering phytohormonal signaling pathways, and similar processes are observed in plants at the early stage of the development of symbiosis (Oldroyd et al., 2009; Pieterse et al., 2012). Mycorrhizal fungi can survive and grow within the host plant, because the defense signaling pathways in the plant are modified in the presence of mycorrhizal fungi (García-Garrido & Ocampo, 2002). Although defense mechanisms, such as the activation of pathogenesis-related genes, can be observed in mycorrhizal roots, these defense responses are weak and not identical to those triggered by interactions with pathogens (Gianinazzi-Pearson, 1996). A good example is the biosynthesis of flavonoids instead of isoflavone reductase, defense-related enzyme, which results in mycorrhizal growth (Harrison, 2005). Colonization of roots by mycorrhizae is sometime called accommodation, because fungi can survive and grow in the host by limiting their defense response (Martin et al., 2017).

While the establishment of symbiosis between mycorrhizal fungi and plants involves the production and perception of hormones causing chemical changes and signaling pathways in both partners, the maintenance of the relationship over both evolutionary and short time periods may be controlled by the host plants. For AM fungi, the spread of hyphae occurs only in the inner cortical cells of the roots, and arbuscules form within the plant cell surrounded by a plant-derived membrane that prevent contact with the root cytoplasm (Parniske, 2008). The limited hyphal proliferation within the roots may be evidence that the host plant controls mycorrhizal growth and thus the interaction with mycorrhizal symbionts (Bais et al., 2006). Moreover,

maintenance of these symbiotic relationships may depend on the supply of nutrients provided by the mycorrhizal symbionts. Plants may allocate carbon to their mycorrhizal symbionts in proportion with the amount of phosphorus and nitrogen AM and ECM fungi provide, respectively, to plants (Bogar et al., 2022; Christian & Bever, 2018).

### **2.3 The importance of host plant-mycorrhizal associations for community structure**

An ecological community is assembled by multiple organisms interacting within and across trophic levels. Although understanding the effects of a single species on a community is an important piece of the puzzle, understanding the effects of organisms at different trophic levels allows broader insights into how the community is structured. Interactions between mycorrhizal fungi and their host plants are expected to have a significant influence on the structure of the broader communities in which they occur. Their influence will vary as a function of: how plants are affected by their symbionts (responsiveness of plants to mycorrhizal fungi), which host species mycorrhizae have an association with (host specificity), the relationship among plants connected through mycorrhizal networks (i.e., cooperation vs. competition), and the nature of host plants-mycorrhizal interactions (i.e., positive vs. negative plant-soil biota feedback). Whether the interaction between plants and fungi is positive or negative may be determined by the stage of ecosystem development (Zangaro et al., 2000a).

Responsiveness of plants to mycorrhizal fungi, called mycorrhizal responsiveness or dependency, is usually defined as the difference in plant growth with and without mycorrhizal fungi (Janos, 2007). The influence of mycorrhizal fungi on plant biomass is mainly, but not always, positive, depending on the biotic composition (e.g., fungal community and the association between plants and fungi) and abiotic environmental conditions (e.g., nutrient

availability and salt level) (Lin et al., 2015; Moora, 2014; van der Heijden & Horton, 2009). For example, a positive response to AM fungi is found in obligately- and facultatively-dependent plants, but not in non-mycorrhizal plants (Lin et al., 2015). The strength of mycorrhizal responsiveness in plants may affect carbon allocation to mycorrhizas and the competition between host plants (Bever et al., 2009; Hartnett et al., 1993). Wild onion *Allium vineale* that significantly increased plant mass in the presence of mycorrhizal species *Scutellospora fulgida* compared to another fungal species (*Glomus claroideum*) presumably allocated higher amounts of photosynthates to the more beneficial symbionts (*S. fulgida*) as indicated by higher spore production (Bever et al., 2009). In this case, mycorrhizal fungi increased plant performance while the host plants also promoted fungal performance, resulting in a positive plant-fungi feedback loop.

Interestingly, high mycorrhizal responsiveness does not necessarily benefit the host plant and may instead lead to strong intraspecific competition. Intraspecific interactions dramatically decreased biomass of the obligately-dependent species, *Andropogon gerardii*, in the presence of mycorrhizal fungi, while the effect of mycorrhizae on intraspecific competition was negligible in less mycorrhizal-dependent *Elymus canadensis* (Hartnett et al., 1993). Generally, an increase in plant growth in the presence of mycorrhizal symbionts is considered to be a positive effect of mycorrhizae. At the same time, however, the increased plant growth may increase overlap among neighboring plants, leading to intense competition for space and resources. Plants in high-density populations would thus experience even stronger competition, and the mycorrhizal effect on plants would be no longer positive in such populations (Hartnett et al., 1993).

Mycorrhizal responsiveness may be linked to ecosystem development as well, as evidenced by a high mycorrhizal responsiveness in early-successional plants that decreases with



the progression of successional stages. The decrease in mycorrhizal responsiveness by plants is often, although not always (Koziol et al., 2015; Middleton & Bever, 2012), associated with nutrient availability (Zangaro et al., 2000a). Since a positive plant-fungi interaction is a common feature at the earlier stage of succession (Dickie et al., 2002c), both plants and fungi may commonly promote growth and abundance each other in early succession. According to a study on pioneer and secondary plants interacting with and without AM fungi, the pioneer *Lafloensia pacari* increased growth rates with AM fungi, but intraspecific competition was not necessarily higher in the presence of AM fungi (Danieli-Silva et al., 2010), resulting in a net positive mycorrhizal effect on the host plants. In contrast, the secondary species, *Cabralea canjerana*, suppressed their growth, and strong intraspecific competition was found with AM fungi indicating the negative mycorrhizal effect on the plants. Although the effect of the host plants on AM fungi was not directly tested in this study, their results revealed that AM fungi positively and negatively affected the pioneer and secondary plant species, respectively.

Positive and negative interactions between host plants and mycorrhizal symbionts may explain the changes observed in plant communities along during ecosystem development. Positive feedback at the early stages of ecosystem development should help both the pioneer plants and mycorrhizas increase their local density (Dickie et al., 2002a; Nara, 2006a). As population densities increase, zones of resource depletion would expand and become more overlapped between plants or mycorrhizas. The mycorrhizal symbionts may eventually reduce the growth of pioneer plants and lead to species replacement during the successional change (Bever et al., 1997). The negative feedback between plants and mycorrhizas may be a key mechanism underlying the maintenance of co-existing species (Bever, 2002; Bever et al., 1997).

Most mycorrhizal fungi are not host specific and can form mycorrhizal networks with multiple plants species, even simultaneously (van der Heijden & Horton, 2009). AM fungi in particular are distributed in a wide range of areas associating with a variety of plant species (Öpik et al., 2006). Nonetheless, AM fungi showed host preferences in previous studies (Öpik et al., 2006). Although higher host specificity in ECM fungi, which are distributed in a narrower range of habitats, is documented relative to that of AM fungi (van der Heijden & Horton, 2009), some studies found a lack of host specificity in ECM fungi (Read, 1991). Mycorrhizas of both classes are capable of forming extensive hyphal networks connecting multiple plant species (van der Heijden & Horton, 2009), exchanging minerals, carbon, and warning signals (Gilbert & Johnson, 2017; Song et al., 2010).

Since mycorrhizal networks can connect multiple individuals of the same and different host-plant species, while plants can be colonized by multiple fungal species, the mycelium network can lead to plant-plant interactions and plant-mycorrhizal interactions. The nature of these interactions among mycorrhizas and plants may depend on the identity of mycorrhizas and plants and the strength of genetic relatedness among the host plants. The presence of neighboring plants with mycorrhizal symbionts may affect plant growth and even their competitive ability since plant biomass may be reduced in the presence of neighbors to which they are likely connected through a mycelium network (Scheublin et al., 2007). In an extreme example, a neighboring plant may be indirectly parasitic such as the case of plants that lack chlorophyll (van der Heijden & Horton, 2009). One plant invests in mycorrhizal growth by allocating photosynthates while another plant benefits by relying on the neighbor for carbon resources and nutrients. However, in such a case, the relationship may not be stable since the

plants detecting the competitive effect of the neighbor, can respond, for example, through reduced carbon allocation to mycorrhizal symbionts (Engelmoer & Kiers, 2015).

In addition to its effect on plant performance, the strength of genetic relatedness between plants connected via mycorrhizal hyphae may also affect mycorrhizal growth. Earlier work suggested that plants that are closely related and cooperative may allocate more photosynthate to mycorrhizal fungi and reduce the formation of pathogen-induced root lesions (File, Klironomos, et al., 2012). Although carbon allocation to mycorrhizal symbionts is costly to host plants, closely related plants sharing a mycelial network can increase mycorrhizal growth as well as plant performance, in a positive plant-fungi feedback loop (File, Klironomos, et al., 2012). In other cases, interacting plants and mycorrhizal fungi have negative fitness consequences. Among two host species (*Panicum sphaerocarpon* and *Plantago lanceolata*) and eight mycorrhizal species, host preference by the fungal species resulted in asymmetrical delivery of host photosynthate to the symbiont species, and the mycorrhizal associations led to uneven increases in growth across the plant species (Bever, 2002). *Panicum* increased the abundance of the fungal species that promoted growth of *Plantago* without increasing *Panicum* growth. Given the importance of negative plant-mycorrhizae feedback for species replacement during the ecosystem development discussed above (see section 2.3), negative feedback between plants and mycorrhizae may also promote the coexistence of multiple plant and mycorrhizal species.

Interactions between mycorrhizas and their host plants may also vary with the degree of dominance in the community in a cause-and-effect manner (van der Heijden & Horton, 2009). The influence of soil composition on plant communities is well documented, where the dominant mycorrhizal species in the soil determine which plant species in the community benefit from their association (Dickie et al., 2002c; Horton et al., 1999). Interestingly, the plant species that

dominate the community do not necessarily receive the most benefit from mycorrhizal symbionts (van der Heijden & Horton, 2009). Increased abundance of the dominant plant species through their association with mycorrhizal fungi may lead to reduced plant diversity in the community (Hartnett & Wilson, 1999). To maintain plant diversity, the mycorrhizal influence on the dominant plant species may be negative while the mycorrhizal influence on the subordinate plants is positive (van der Heijden et al., 1998; Zhang et al., 2014). Based on the evidence that a change in the mycorrhizal composition can lead to decreased dominance of plant species (Stampe & Daehler, 2003), the composition of mycorrhizal species may play a prominent role in maintaining balance within the plant community.

In summary, growth of both plants and mycorrhizal fungi is influenced by a complex interaction between host plants and their mycorrhizal symbionts. Although interaction with mycorrhizal fungi tends to increase plant growth, the responsiveness of plants to mycorrhizal fungi varies with plant species, plant density, and local environmental conditions. Some mycorrhizal fungi are host specific, but many can colonize multiple plant species, and all are capable of connecting multiple plants through mycelial networks, thus promoting plant-plant interactions. Plant-plant interactions ranging from cooperation to competition can affect not only the performance of the plants themselves but also the growth of their mycorrhizal symbionts. The relationship between plants and mycorrhizal fungi may be positive or negative, depending on plant density and the successional stage of the ecological community. The nature of the plant-fungi feedback can affect the abundance of both the plant and mycorrhizal species and play an important role in shaping the broader ecological community.

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### **3. Plant-plant interactions in early-successional (*Metrosideros polymorpha* var. *incana*) and late-successional (*M. polymorpha* var. *glaberrima*) varieties with mycorrhizal fungi**

#### **3.1 Introduction**

Plant-plant interactions play an important role in structuring plant communities. Genetic relatedness among neighboring plants can affect their interactions positively or negatively through cooperation or competition (Cheplick & Kane, 2004; Dudley & File, 2007). In general, competing plants tend to decrease growth as a result of resource depletion, while cooperative (i.e., facilitative) plants may either increase or decrease their growth rate in response to neighbors (Armas et al., 2004). A complication of plant interaction studies thus arises from the challenge of distinguishing between cooperation and competition when small plant size is observed. Additionally, mycorrhizal fungi, which have a mutualistic relationship with most plant species, may have complex effects on plant interactions through their connection with plant roots.

The ability to distinguish kin from non-kin, known as kin recognition, affects social behavior (Penn & Frommen, 2010). Plants may allocate resources differently depending on the genetic relatedness of their neighbors, and kin recognition may therefore help individuals improve their fitness (Chen et al., 2012). In a competitive environment, plants deplete resources from others and suppress plant growth of neighboring individuals (Armas et al., 2004). According to niche-partitioning theory, distantly related plants have different strategies for growth (e.g., short vs. long roots) and survivorship, which allows coexistence by reducing competition. In contrast, closely related plants have similar strategies of resource acquisition, which leads to intense competition where they co-occur (Cheplick & Kane, 2004).



Kin selection favors altruistic behavior towards relatives (Hamilton, 1964), which can appear as restricted growth of neighboring plants that are closely related. By avoiding the cost of competition among close relatives, individuals can share limited resources and benefit from a fitness that is higher than it would be if growing alongside strangers. Therefore, small body size observed in closely related neighboring plants may reflect either competitive or cooperative behavior. Measures of fitness such as reproductive success may help to distinguish between competition and cooperation since niche partitioning increases fitness with distantly related individuals whereas kin selection increases inclusive fitness of closely related individuals (Cheplick & Kane, 2004; File, Murphy, et al., 2012; Murphy & Dudley, 2009). When only plant growth is measured to understand plant-plant interaction, caution is required in the interpretation of the results.

A plant's ability to recognize neighboring genetic relatedness may extend to the population and species levels (Lee et al., 2020). Although cooperative behavior may be observed in interactions with relatives, cooperation may be less common in interactions with more distantly related plants of the same species. At the species level, long-distance dispersal may be favored to minimize conspecific interactions and the negative effects of species-specific predation or parasitism (Connell, 1971; Janzen, 1970; Onguene & Kuyper, 2002). In addition, one species may have the ability to outperform others as observed in invasive plant species that increase biomass more rapidly than native species (Lee et al., 2020). Some plants may even change their competitive ability depending on whether intra- or interspecific interactions are involved. For example, *Plantago lanceolata*, which usually has low root density in an intraspecific interaction, is not necessarily a strong competitor (Padilla et al., 2013). When *P. lanceolata* interacted with *Festuca rubra*, which is known to be a strong competitor having high

root density in an intraspecific interaction, *P. lanceolata* increased root density and became a dominant species. Interestingly, the enhanced competitive ability in *P. lanceolata* may be short lived, since *F. rubra* became dominant later. In sum, the behavior of neighboring plants may vary as a function of the strength of their relationship, from siblings to different species.

Almost all land plant species have a mutualistic relationship with mycorrhizal fungi that physically interact with plant roots. These fungi can increase plant growth by providing nutrients (e.g., phosphorus and nitrogen) as well as increase plant resistance to biotic and abiotic stress (Gorzelak et al., 2015). In turn, plants provide photosynthetic products (e.g., carbohydrates) and shelter for the mutualistic microbes, increasing their growth and reproduction (S. E. Smith & Read, 2008). Additionally, the mycorrhizal fungal network can serve as a conduit between neighboring plants, transferring not only mineral nutrients but also signals from one plant to another (Babikova et al., 2013; S. E. Smith & Read, 2008).

Mycorrhizal effects on the host plants are typically positive by increasing nutrient uptake, plant growth, and survival, but may lead to negative consequences under some conditions (Babikova et al., 2013; Lambers, Raven, et al., 2008; Simard, 2018). The increased plant size in the presence of mycorrhizal symbionts may lead to increased competition by overlap between neighboring plants for resource acquisition, especially in high-density populations. Therefore, the effects of mycorrhizae on plants are expected to be less positive in high-density populations (Hartnett et al., 1993). From the plant perspective, how much plants change their growth in response to mycorrhizal symbionts may vary depending on biotic and abiotic environmental conditions (Lin et al., 2015; Moora, 2014; van der Heijden & Horton, 2009). For example, mycorrhizal responsiveness tends to be high in early-successional plants, decreasing progressively as the successional stage progresses, probably in association with changes in

nutrient availability (Zangaro et al., 2000a). Both plants and fungi may promote the growth and abundance of each other in early-successional stages, where positive plant-fungi feedback is common (Dickie et al., 2002b). At later successional stages, in contrast, mycorrhizal fungi may lead to strong intraspecific competition and a net negative effect on plants (Bever et al., 1997; Danieli-Silva et al., 2010). Therefore, the shift from positive to negative frequent-dependent feedback may be seen during ecological development, leading to the coexistence of multiple plant species at later successional stages (Dickie et al., 2013).

The Hawaiian dominant tree species, *Metrosideros polymorpha*, is distributed across a wide range of habitats including wet and dry forests and young and old substrates (Corn, 1972). *M. polymorpha* var. *incana* (*incana* hereafter), an early-successional variety, can colonize four year-old lava flows below ~1,200 m above sea level (Smathers & Mueller-Dombois, 1974). Over time (1,400 – 3,000 years), *incana* is replaced with the late-successional variety, *M. polymorpha* var. *glaberrima* (*glaberrima* hereafter) (Drake & Mueller-Dombois, 1993; Kitayama et al., 1995). The two successional varieties can occur sympatrically or parapatrically, and easily hybridize where their ranges overlap (Zangaro et al., 2000b). Even though gene exchange frequently occurs between these varieties, they maintain significant morphological, ecological, and genetic differences (DeBoer & Stacy, 2013; Morrison & Stacy, 2014). Light, water, and nutrient availability are likely important drivers of differentiation between these varieties (Hoof et al., 2008; Morrison & Stacy, 2014; Stemmermann, 1983; Vitousek et al., 1995). The early-successional *incana*, which is naturally found on young, open, dry, nutrient-limited (nitrogen and inorganic phosphate) substrates, has better performance under high light than *glaberrima*, which dominates forests on mature, nutrient-rich substrates (Morrison & Stacy, 2014).

The low population density of incana on the open substrates of new lava flows implies a low probability of incana interacting with other plants. On the other hand, glaberrima, occurring in late-successional forests with high plant densities, is expected to interact frequently with other plants. Moreover, mycorrhizal abundance is expected to increase along successional gradients in Hawaii (Gemma et al., 1992). Rates of root colonization by mycorrhizae increase with increasing substrate ages (Gemma & Koske, 1990), and mycorrhizal responsiveness in plants is expected to decrease with succession (Zangaro et al., 2000b). Given that incana and glaberrima differ in population density and the abundance of mycorrhizal fungi in their respective early- and late-successional environments, these two varieties are ideally suited to look for evidence of kin recognition and the importance of mycorrhizae in plant interactions. This research aims to compare the nature of plant-plant interactions between the early- and late-successional varieties, incana and glaberrima, respectively of *M. polymorpha*. The questions to be addressed are:

1. Do mycorrhizal fungi affect the growth of *M. polymorpha* seedlings?
2. Do *M. polymorpha* seedlings allocate resources differently according to their genetic relationship with their neighbor (i.e., sibling, different population, or different variety)?
3. Do mycorrhizal fungi facilitate kin recognition in *M. polymorpha* seedlings?
4. Does the strength of kin recognition differ between incana and glaberrima?

I predict that seedlings will increase growth as the genetic relatedness with the neighboring seedling increases and the pattern of response to the neighbors will differ both between the two varieties and in the presence/absence of mycorrhizae.

## 3.2 Materials and methods

### 3.2.1 Seed collection and germination

Open-pollinated seeds from 5-10 mature fruit capsules were collected from each of approximately 25 trees of *Metrosideros polymorpha* from each site (population). The seeds of *incana* were sampled at Ka'ū Desert located in Hawai'i Volcanoes National Park and Mohouli street in Hilo. The seeds of *glaberrima* were collected at Saddle Road MM14 and Stainback Highway. Trees of two varieties were identified based on morphological characteristics: *incana* has pubescent leaves on new substrates, while *glaberrima* has glabrous leaves. To minimize the impact of natural hybridization on this study, *incana*-*glaberrima* hybrids, which typically possess caducous (removable by rubbing) leaf pubescence (Stacy et al., 2016), were avoided. All fruit capsules were stored in coin envelopes at room temperature until used. The soil mix for the experiment was prepared with 3:2:1:1 of low-nutrient medium (Sun Gro Horticulture Sunshine Mix #1), cinder, perlite, and ash soil collected from the Hamakua coast. Ash soil was added to change phosphorus to a form unavailable for plants (Brady & Weil, 2004; United States. Soil Conservation Service, 1973). For the treatment with mycorrhizae, 3.5 tablespoons of a commercially available mycorrhizal fungi mix including seven arbuscule mycorrhizal (AM) fungi species (*Glomus intraridices*, *G. mosseae*, *G. etunicatum*, *G. clarum*, *G. monosporum*, *G. deserticola*, and *Gigaspora margarita*) and one ectomycorrhizal (ECM) fungi species (*Pisolithus tinctorius*) were added to the soil mix and homogenized well. The soil mix was packed into 9.5 cmx12.2-cm communal wells and covered with black sand, which allowed visualization of the germinated seeds. Seeds were sown at a rate of approximately three fruit capsules per two wells. Trays were misted every 30 min for 20 sec (daytime only) in a mist house at Pana'ewa Farm at the University of Hawai'i Hilo.

### 3.2.2 Greenhouse experiment

After germination, seedlings (~ 0.5 cm tall) were transferred in pairs to individual 5 x 5.5-cm pots, each containing one target seedling and one neighbor seedling. The target seedlings were split evenly between *incana* and *glaberrima* and were allocated among four treatments according to the genetic relatedness of the neighbor to the target: alone (see below), sibling (sib), pop (neighbor derives from a different population of the same variety), and var (neighbor is of the opposite variety). For the “alone” treatment, the soil in the pot was divided evenly in half with a plastic divider. This was done to ensure roughly uniform soil availability across target seedlings regardless of treatment. The seedlings were grown for ~15 months in a greenhouse (approximately 800  $\mu\text{mol}/\text{m}^2/\text{s}$ ) with overhead water three times per day for five minutes at the University of Hawai‘i Hilo’s Pana‘ewa Farm at ~100 m in elevation. After 15 months of growth, all target and neighbor seedlings were removed from their pots, and final aboveground and belowground plant traits were measured. Measurements included shoot length (mm), root length (mm), total leaf area (using a LI-COR 3100 leaf area meter; LI-COR Inc., Lincoln, NE, USA), total shoot dry mass (mg), total root dry mass (mg), and total leaf dry mass (mg). Dry masses were recorded after seedlings were dried at 70 °C for at least 48 hours. For each seedling, the following calculations were done:

$$\text{Total dry mass (TDM, mg)} = \text{shoot dry mass (mg)} + \text{root dry mass (mg)}$$

$$\text{Specific leaf area (SLA, mm}^2/\text{mg)} = \text{total leaf area (mm}^2) / \text{leaf dry mass (mg)}$$

$$\text{Root:shoot length ratio} = \text{root length (mm)} / \text{shoot length (mm)}$$

$$\text{Root:shoot mass ratio} = \text{root mass (mg)} / \text{shoot mass (mg)}$$

In addition, mycorrhizal responsiveness was calculated to reveal how much seedlings changed their growth in response to the presence of mycorrhizal symbionts (Miyasaka et al., 1993):

$$\text{Mycorrhizal responsiveness (\%)} = \frac{(\text{TDM with mycorrhizal symbionts}) - (\text{TDM without symbionts})}{\text{TDM with symbionts}} \times 100$$

### 3.2.3 Statistical analyses

The effects of variety (incana or glaberrima), treatment (alone, sib, pop, or var), mycorrhizal fungi (presence or absence), and interactions among these three factors on target seedling growth were analyzed using generalized linear model (GLMs) with a gamma distribution in R ver.4.0.5 (R Development Core Team, 2001). The analyses were then repeated for each of the target seedlings of each variety separately. To understand the effect of neighboring seedling size on target seedling growth, each measure of neighbor size was added in turn to the GLMs as a covariate.

All analyses were done using package ‘lme4’ in R version 3.1.2 (RCoreTeam 2012; Bates et al. 2014). Parameter significance was estimated using Satterthwaite approximation with package ‘lmerTest’ (Kunetsova et al. 2013).

In addition, in a separate analysis, the effect of neighbor TDM on the target TDM was estimated using ANOVA. The continuous variable, neighbor TDM, was converted to a categorical variable by grouping neighbors into small or large TDM categories (0.0101 – 0.1616 mg or 0.1644 – 1.3603 mg, respectively). Seedlings were divided roughly in half between these groups.

### 3.3 Results

#### 3.3.1 Effects of mycorrhizal fungi on seedling growth in *M. polymorpha* var. *incana* and *M. polymorpha* var. *glaberrima*

Seedling growth was not significantly affected by the 3-way interaction term (myco x treatment x variety) or the 2-way interaction term (myco x variety). However, a significant positive effect of mycorrhizal fungi was observed on all seven growth measurements of seedlings (Fig. 3.1). For example, total dry mass (TDM) of seedlings was significantly greater in pots with mycorrhizal fungi compared to those without mycorrhizae ( $t = -8.77$ ,  $df = 232.25$ ,  $p < 0.001$ ). Mycorrhizal dependency calculated based on TDM was 62.5 % overall (Fig. 3.2). A greater mycorrhizal dependency was observed in *incana* (65.8 %) than *glaberrima* (59.3 %).



Variable	Total Dry Mass			Shoot Dry Mass			Root Dry Mass			Shoot Length			Root Length			Total Leaf Area			Total Leaf Mass			SLA			R:S Mass Ratio			R:S Length Ratio			Myco		
	Chisq	Df	p-value	Chisq	Df	p-value	Chisq	Df	p-value	Chisq	Df	p-value	Chisq	Df	p-value	Chisq	Df	p-value	Chisq	Df	p-value	Chisq	Df	p-value	Chisq	Df	p-value	Chisq	Df	p-value			
Myco x Trtmt x Var	2.46	3	0.483	3.47	3	0.325	1.19	3	0.755	0.35	3	0.95	0.98	3	0.806	2.99	3	0.393	3.05	3	0.384	5.76	3	0.124	4.17	3	0.244	0.75	3	0.862			
Myco x Trtmt	1.43	3	0.700	1.26	3	0.74	3.01	3	0.391	2.78	3	0.427	1.71	3	0.635	3.42	3	0.332	2.73	3	0.436	17.88	3	< 0.001	2.54	3	0.468	1.34	3	0.72			
Myc x Var	0.02	1	0.881	0.26	1	0.609	0.00	1	0.96	0.03	1	0.855	0.00	1	0.99	0.04	1	0.849	0.12	1	0.725	1.22	1	0.27	1.37	1	0.241	0.61	1	0.433			
Trtmt x Var	3.02	3	0.389	4.27	3	0.234	3.04	3	0.385	1.31	3	0.727	9.75	3	0.021	2.89	3	0.409	4.24	3	0.237	0.98	3	0.807	0.22	3	0.975	7.92	3	0.048	1.29	3	0.733
Trtmt x Nhbr	9.45	3	0.024	3.37	3	0.338	12.75	3	0.005	10.56	3	0.014	19.27	3	< 0.001	3.37	3	0.338	3.33	3	0.343	4.01	3	0.26	1.76	3	0.623	0.73	3	0.867			
Myco	106.76	1	< 0.001	88.47	1	< 0.001	112.56	1	< 0.001	69.68	1	< 0.001	31.00	1	< 0.001	77.56	1	< 0.001	74.78	1	< 0.001	3.34	1	0.068	1.00	1	0.3182	10.04	1	0.002			
Trtmt	1.50	3	0.683	0.58	3	0.902	4.83	3	0.185	0.38	3	0.945	1.32	3	0.724	0.37	3	0.946	0.63	3	0.889	14.28	3	0.003	3.67	3	0.3	0.38	3	0.944	10.00	3	0.019
Var	5.51	1	0.019	7.13	1	0.008	3.10	1	0.079	16.27	1	< 0.001	6.06	1	0.014	17.47	1	< 0.001	7.25	1	0.007	12.39	1	< 0.001	1.34	1	0.247	3.73	1	0.053	4.57	1	0.033
Nhbr	11.71	1	0.001	7.59	1	0.006	10.28	1	0.001	0.01	1	0.907	0.01	1	0.944	15.24	1	< 0.001	10.04	1	0.002	1.39	1	0.238	1.81	1	0.179	9.03	1	0.003			

Fig. 3.1 Results of full generalized linear model of growth measurements of *M. polymorpha* seedlings after ~15 months of the plant-plant-interaction experiment. Myco = presence or absence of mycorrhizae, Trtmt = genetic relatedness of neighboring seedling to target seedling, Var = variety of the target seedling, and Nhbr = size of the neighboring seedling.

<b>Mycorrhizal fungi</b>	<b>All</b>	<b>I</b>	<b>G</b>
with	0.32 (185)	0.38 (94)	0.27 (91)
without	0.12 (170)	0.13 (90)	0.11 (80)
<b>Mycorrhizal responsiveness</b>	<b>62.5%</b>	<b>65.8%</b>	<b>59.3%</b>

Fig. 3.2 Average total dry mass (mg) of target seedlings of *M. polymorpha* grown for 15 months with and without mycorrhizal fungi with target varieties and treatments pooled. Mycorrhizal responsiveness indicates how much *M. polymorpha* changed total dry mass in response to the presence of mycorrhizal fungi. ( ) = number of samples.

The value of the root:shoot length ratio was greater than one in *M. polymorpha* seedlings both with and without mycorrhizal fungi in the soil, indicating that seedlings allocated more to root length than shoot length in general. In the absence of mycorrhizal fungi, a significant increase in allocation to root length relative to shoot length was observed ( $t = 3.49$ ,  $df = 310.48$ ,  $p < 0.001$ ; Fig. 3.3). When *incana* and *glaberrima* were analyzed separately, the pattern of greater root:shoot length ratio in the absence of mycorrhizal fungi was observed in both varieties (*incana*:  $t = 2.10$ ,  $df = 164.46$ ,  $P = 0.037$ ; *glaberrima*:  $t = 2.93$ ,  $df = 144.56$ ,  $p = 0.004$ ; Fig. 3.4). Interestingly, there was no effect of mycorrhizal fungi on root:shoot mass ratio ( $t = -0.90$ ,  $df = 347.17$ ,  $p = 0.369$ ).

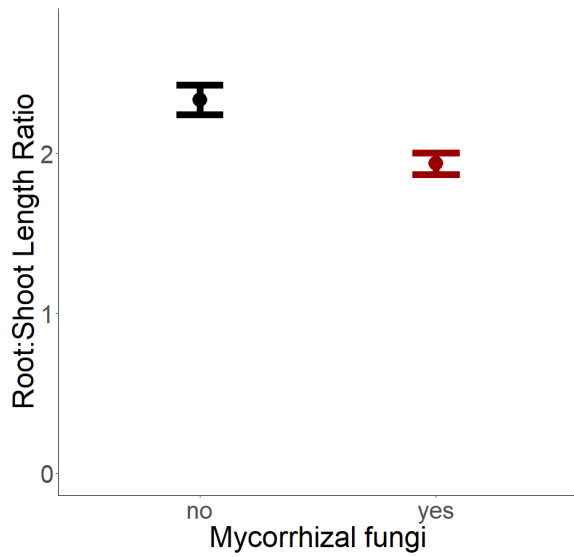


Fig. 3.3 Mean  $\pm$  SE root:shoot length ratio of seedlings of *Metrosideros polymorpha* with (red) and without (black) mycorrhizal fungi grown for  $\sim$ 15 months in a greenhouse with both varieties and treatments pooled.

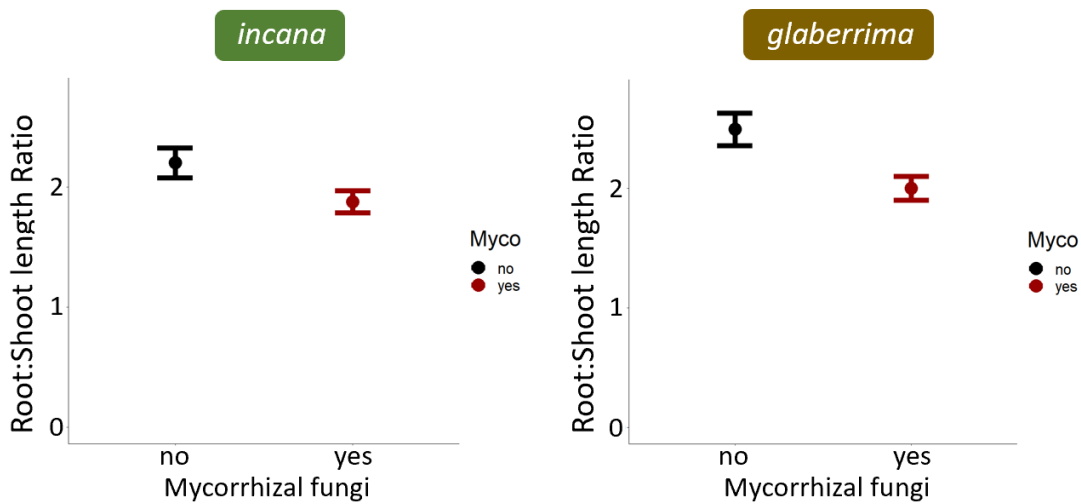


Fig. 3.4 Mean  $\pm$  SE root:shoot length ratio of seedlings in *incana* and *glaberrima* with (red) and without (black) mycorrhizal fungi grown for  $\sim$ 15 months in a greenhouse with varieties and treatments pooled.

### 3.3.2 Difference in seedling growth between incana and glaberrima

Six of seven growth measurements demonstrated statistically higher growth rates in incana than in glaberrima. For example, incana had significantly larger TDM than glaberrima ( $t = 2.53$ ,  $df = 346.60$ ,  $p = 0.012$ , Fig. 3.5). Only root dry mass showed a non-significant increase in incana compared to glaberrima. Further, when the two varieties were grown together in the same pot in the var treatment, incana seedlings were always larger than glaberrima seedlings.

There was no significant influence of the interaction between variety and treatment on root:shoot mass ratio ( $\chi^2 = 0.22$ ,  $df = 3$ ,  $p = 0.975$ ). As a general pattern, root:shoot mass ratio was close to one or below one for all treatments, indicating relatively greater allocation to shoot mass than root mass (Fig. 3.6). Although root:shoot mass ratio was not affected by the interaction between variety and treatment, a significant effect of the interaction between variety and treatment on root:shoot length ratio was observed ( $\chi^2 = 7.92$ ,  $df = 3$ ,  $p = 0.048$ ). The root:shoot length ratio was similar between alone and sib treatments and between stranger treatments (i.e., the pop and var) for each of incana and glaberrima (Fig. 3.7). The four means of root:shoot length ratios of the two stranger treatments in each of incana and glaberrima were also similar to each other. A difference in root:shoot length ratio was observed when alone/sib and pop/var treatments were compared in both varieties with relatively greater allocation to root length in the alone/sib treatments in glaberrima, and lower allocation to root length in the alone/sib treatments in incana.

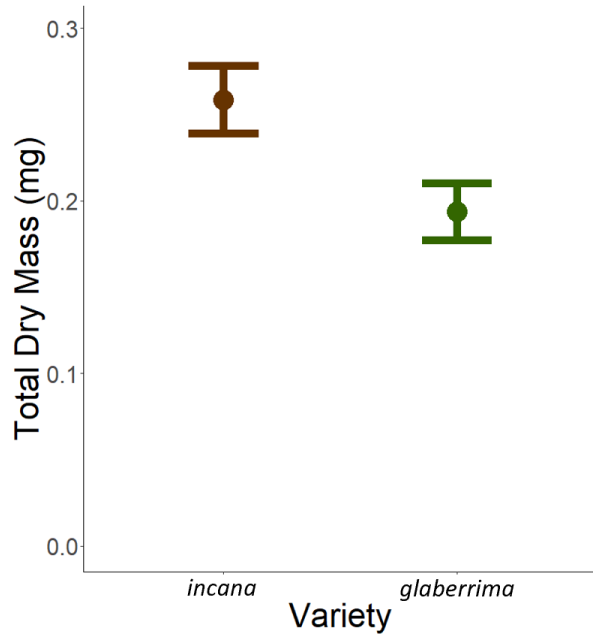


Fig. 3.5 Mean  $\pm$  SE total dry mass (mg) of *M. polymorpha* var. *incana* (brown) and var. *glaberrima* (green) grown alone, with a sibling, with a different population within the same variety, or with the opposite variety for ~15 months in a greenhouse with and without mycorrhizae pooled.

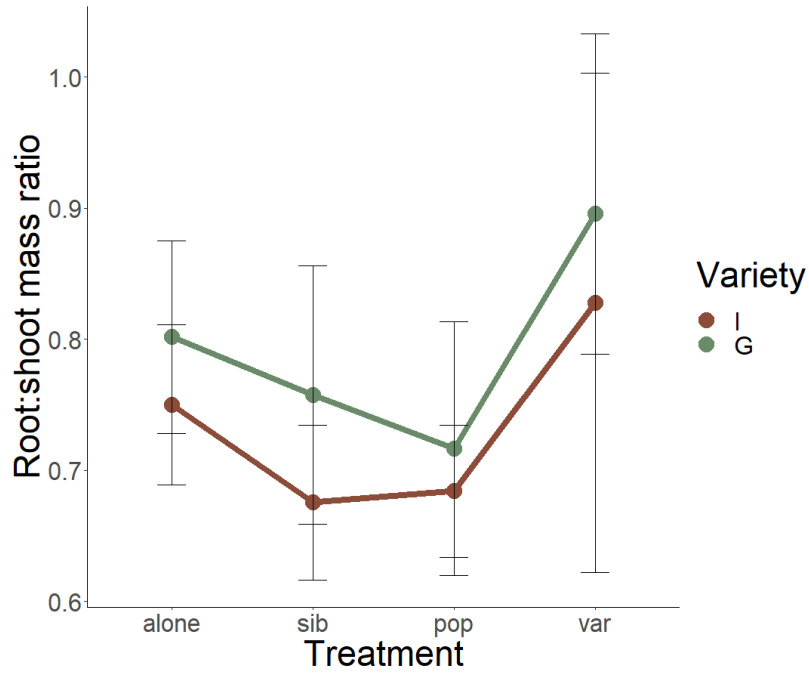


Fig. 3.6 Mean  $\pm$  SE root:shoot mass ratio of *Metrosideros polymorpha* var. *incana* (brown) and var. *glaberrima* (green) grown alone, with a sibling, with a different population within the same variety, or with the opposite variety for ~15 months in a greenhouse with and without mycorrhizae pooled.

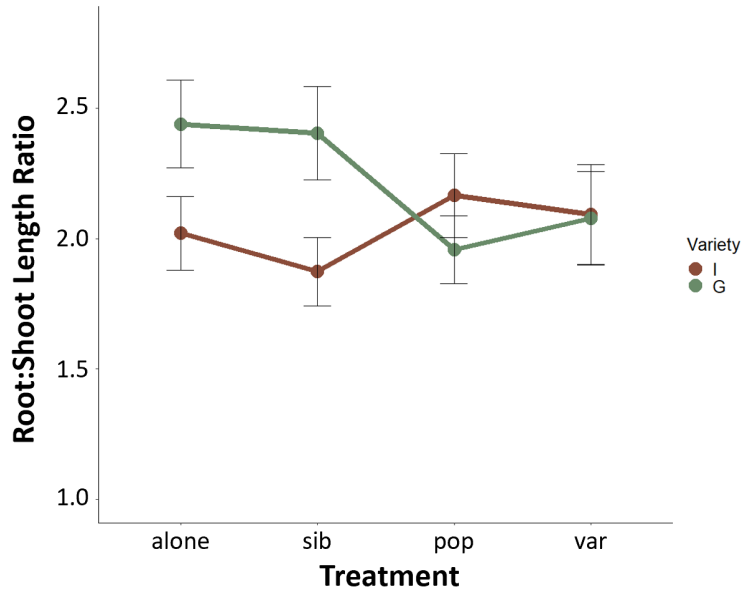


Fig. 3.7 Mean  $\pm$  SE root:shoot length ratio of *Metrosideros polymorpha* var. *incana* (brown) and var. *glaberrima* (green) grown alone, with a sibling, with a different population within the same variety, or with the opposite variety for ~15 months in a greenhouse with and without mycorrhizae pooled.

Peak SLA was observed in the sibling treatment in the absence of mycorrhizal fungi (Myco x Trtmt Interaction:  $\chi^2 = 17.88$ ,  $df = 3$ ,  $p < 0.001$ ). This pattern was observed whether target seedlings of the two varieties were analyzed together or separately. Yet, the significant difference in SLA was observed in the combined analysis (both varieties) and in *glaberrima* alone, but not in *incana* alone (Fig. 3.8, Fig. 3.9, Fig. 3.10).

Variable		<i>Chisq</i>	<i>Df</i>	<i>p-value</i>
All	Myco x Trtmt x Var	5.763	3	0.124
	Myco x Trtmt	17.881	3	< <b>0.001</b>
	Myc x Var	1.216	1	0.270
	Trtmt x Var	0.978	3	0.807
	Trtmt x Nhbr size	4.010	3	0.260
	Myco	3.341	1	0.068
	Trtmt	14.278	3	<b>0.003</b>
	Var	12.394	1	< <b>0.001</b>
	Nhbr size	1.394	1	0.238
incana	Myco x Trtmt	5.847	3	0.119
	Trtmt x Nhbr size	0.892	3	0.827
	Myco	0.86	1	0.354
	Trtmt	7.446	3	0.059
	Nhbr size	2.469	1	0.116
glaberrima	Myco x Trtmt	13.505	3	<b>0.004</b>
	Trtmt x Nhbr size	3.866	3	0.276
	Myco	3.105	1	0.078
	Trtmt	7.664	3	0.053
	Nhbr size	0.082	1	0.774

Fig. 3.8 Results of generalized linear models testing variation in SLA of seedlings grown alone, with a sibling, with a different population within the same variety, or with the opposite variety in the presence or absence of mycorrhizal fungi for ~15 months in a greenhouse. Results are shown for all target seedlings pooled, incana target seedlings, and glaberrima target seedlings. Myco = presence or absence of mycorrhizae, Trtmt = genetic relatedness of neighboring seedling to target seedling, Var = variety of the target seedling, Nhbr size = the size of the neighboring seedling.



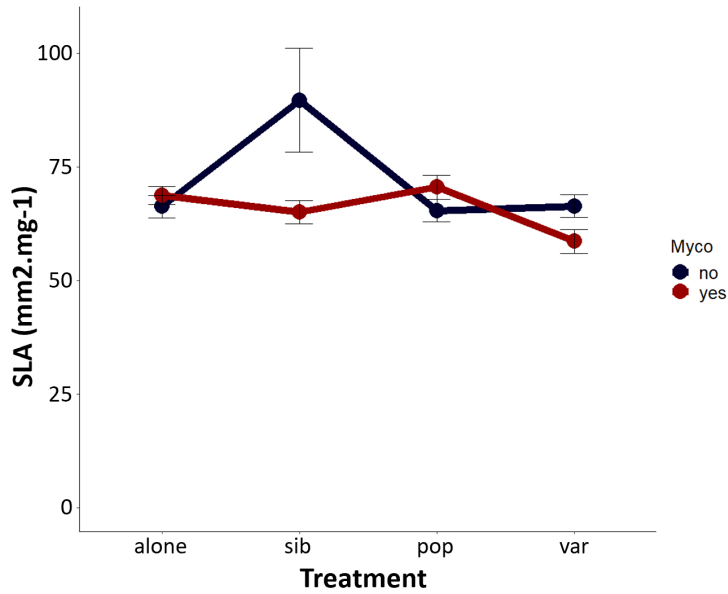


Fig. 3.9 Mean  $\pm$  SE SLA of seedlings in *Metrosideros polymorpha* with (red) and without mycorrhizal fungi (black) grown alone, with a sibling, with a different population within the same variety, or with the opposite variety in the presence and absence of mycorrhizal fungi for ~15 months in a greenhouse with varieties pooled.

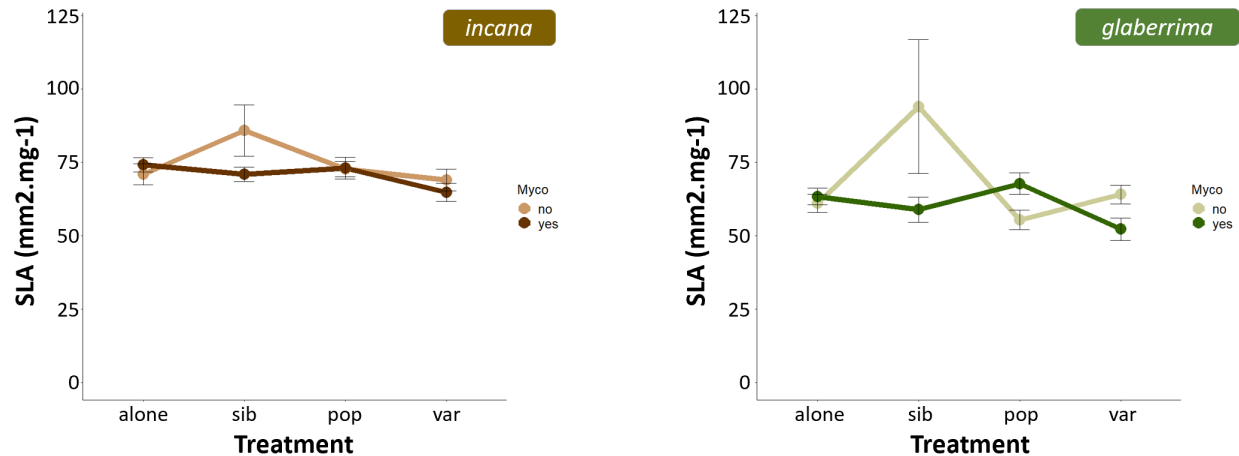


Fig. 3.10 Mean  $\pm$  SE SLA of seedlings in *Metrosideros polymorpha* var. *incana* (brown) and var. *glaberrima* (green) with (darker) and without mycorrhizal fungi (lighter) grown alone, with a sibling, with a different population within the same variety, or with the opposite variety for ~15 months in a greenhouse.

### 3.3.3 The effect of neighbor seedling size on growth of the target seedling

The size of the neighbor seedling significantly affected the dry mass and leaf traits of the target seedlings (Fig. 3.1). The significant interaction effect between neighbor size and treatment was detected for total dry mass, root dry mass, shoot length and root length, for which the sizes of the target and neighboring seedlings were inversely correlated. In contrast to this general trend, for *glaberrima* target seedlings only, when the neighbor seedling was a sibling, the neighbor size had a positive effect on the size of the target seedling regardless of presence or absence of mycorrhizal fungi (Fig. 3.11). An analysis of the relationship between the target size and neighbor size of siblings clearly showed that the neighbor TDM positively affected the target TDM in the sib treatment in *glaberrima* but not in *incana* (neighbor size x variety;  $F = 4.57$ ,  $p = 0.035$ ; Fig. 3.12).

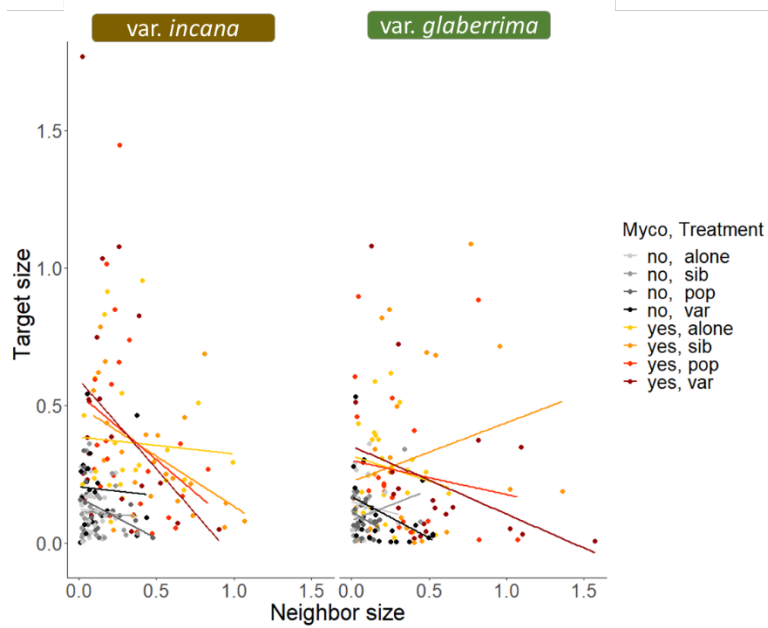


Fig. 3.11 The scatterplot of target and neighbor size in terms of total dry mass in *Metrosideros polymorpha* var. *incana* and var. *glaberrima* with (warm color) and without mycorrhizal fungi (gray) grown alone, with a sibling, with a different population within the same variety, or with the opposite variety for ~15 months in a greenhouse.

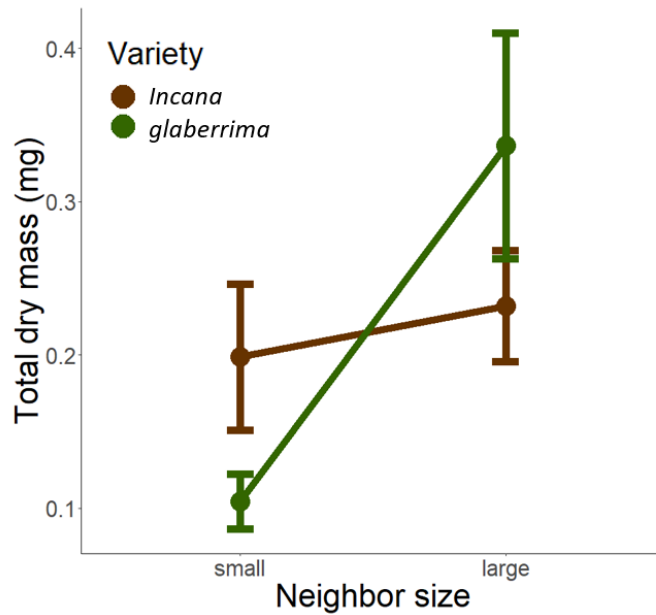


Fig. 3.12 The effect of neighbor size (small vs. large) on the size (total dry mass) of target seedlings in *Metrosideros polymorpha* var. *incana* (brown) and var. *glaberrima* (green) grown for ~15 months in a greenhouse with and without mycorrhizae pooled and all treatments pooled.

### 3.4 Discussion

#### 3.4.1 The effect of mycorrhizae on seedling growth in *M. polymorpha*

The majority of plant species have mutualistic relationships with mycorrhizal fungi, and some species even have dual infections with AM and ECM fungi (Dickie et al., 2013). The positive effects of mycorrhizal fungi on plants are well-documented, including increased growth, survival, and resistance to biotic and abiotic resistance (Babikova et al., 2013; Li et al., 2006; Onguene & Kuyper, 2002; Vicente-Sánchez et al., 2014). The current study adds to the evidence that mycorrhizal fungi increase seedling growth rates of two successional varieties of Hawaii's landscape-dominant tree, *M. polymorpha*. It is not clear which mycorrhizal species are more

responsible for enhancing seedling growth since a mix of mycorrhizal species including seven AM and one ECM fungal species was used in this study. It is possible that a single fungal species is largely responsible for the effect in *M. polymorpha* or that different fungal species affect *incana* and *glaberrima* differently.

This is the third study to show the positive influence of mycorrhizal fungi on endemic Hawaiian tree species, following studies on mamane (*Sophora chrysophylla*) and koa (*Acacia koa*) (Miyasaka et al., 1993). Based on the categories of plant responsiveness to AM fungi suggested by Habte & Manjunath (1991), mamane is highly responsive to AM fungi, while koa is moderately responsive to AM fungi. These categories of mycorrhizal responsiveness are based on how much plants change their growth rates in response to mycorrhizal symbionts at low and high phosphorus concentrations. Since the phosphorus concentration was not measured in the current study, these categories may not be directly applicable. Nonetheless, given that species with 50-75 % of mycorrhizal responsiveness at a phosphorus concentration of 0.02 mg/L are considered highly responsive, the mycorrhizal responsiveness of *M. polymorpha* observed in the current study (62.5 %) appears to be high.

Increased nutrient uptake and growth rate in plants are well known effects of mycorrhizal fungi. Although plant biomass is often enhanced in the presence of mycorrhizae, it is not always true that belowground growth is increased (Tawaraya, 2003). Because mycorrhizal fungi may aid nutrient acquisition in plants, plants may allocate less biomass to root length in the presence of mycorrhizae, thus reducing the carbon-cost of nutrient acquisition (Ven et al., 2019). In this study, lower allocation to root length than shoot length was observed in the presence of mycorrhizae. Given that root growth may have an inverse relationship with responsiveness to

mycorrhizae (Tawaraya, 2003), the reduced allocation to root length with mycorrhizae may be consistent with relatively high mycorrhizal dependency in *M. polymorpha*.

### **3.4.2 Contrasting responses of two successional varieties of *M. polymorpha* to the presence of mycorrhizal fungi**

Comparison of the two varieties revealed that seedlings of the early-successional incana had a higher total dry mass than those of the late-successional glaberrima regardless of the presence of mycorrhizal fungi. However, incana also showed greater responsiveness to mycorrhizal symbionts (65.8 %) than did glaberrima (59.3 %). The greater responsiveness of incana to mycorrhizal fungi may be associated with the low nutrient availability in early-successional environments (Zangaro et al., 2003). The higher growth rate in incana than glaberrima observed both with and without mycorrhizal fungi may simply reflect the higher growth rate commonly seen in early-successional plants (Grime, 1977; Koziol et al., 2015). Notably, in the var treatment in this study - in which both varieties were grown together in each pot – all measurements consistently demonstrated a smaller size of glaberrima relative to incana, suggesting that incana may be a stronger competitor than glaberrima.

The difference in growth between incana and glaberrima seedlings may be explained by the different strategies of nutrient allocation between incana and glaberrima, in addition to different responsiveness in plants to mycorrhizal fungi stated in Chapter 3. Based on the previous study, an increase in nutrient availability by fertilization affected *M. polymorpha* differently on the young substrate on Hawaii Island and the old substrate on Kauai demonstrating a morphological change by increasing leaf size without foliar nitrogen or chlorophyll contents on the young substrate whereas physiological changes by increasing the photosynthetic rate per the

unit leaf surface area and foliar nitrogen and chlorophyll contents with no change in leaf size on the old substrate (Cordell et al., 2001). If the difference in nutrient allocation between *M. polymorpha* on the young and old substrates demonstrated in the study is also true for early-successional *incana* on the young substrate and late-successional *glaberrima* on the old substrate, the increased nutrient loading to seedlings in the presence of mycorrhizal fungi may or may not lead to an increase in seedling size. Since biomass mainly affected by the photosynthetic products including carbon, hydrogen, and oxygen (Hofius & A. J. Börnke, 2007), carbon allocation could be one of the most important factors determining plant biomass. Under conditions of high nutrient loading with mycorrhizal fungi, *incana* may respond morphologically by increasing carbon allocation to seedling growth, while *glaberrima* may have physiological changes without clear morphological changes.

The contrasting responsiveness of *incana* and *glaberrima* to mycorrhizae may reflect differential local adaptation to the low- and high-population densities of trees in early- and late-successional environments, respectively. *Incana* has a lower likelihood of interacting with other plants on the young open substrates of new lava flows, while *glaberrima* occurs in high-density wet-forest communities. If it is true that biomass and fitness are tightly linked (Aschehoug et al., 2016), high biomass may indicate high fitness. For low-density populations of *incana*, faster seedling growth in the presence of mycorrhizal fungi would not likely increase competition for resources among neighbors; thus, the increased biomass would likely result in increased fitness. For high-density populations of *glaberrima*, in contrast, increased seedling growth rates in the presence of mycorrhizal fungi would not likely result in increased fitness due to the greater likelihood of increased competition with neighbors. According to theory, abundance and fitness are inversely related in a negative frequency-dependent relationship (Bever et al., 1997). As

positive feedback is a common feature in early-successional environments (Dickie et al., 2002b), *incana* and its mycorrhizal symbionts may benefit from a positive-feedback relationship, whereas the more constrained response of late-successional *glaberrima* to mycorrhizae may promote the coexistence of mixed plant species at higher densities.

### **3.4.3 Adaptation to recognize and/or respond to genetically different neighbors in *glaberrima***

The ability to recognize the genetic relatedness of neighbors should help plants to allocate resources to cooperative versus competitive behavior, adjust territories, and promote the fitness of relatives (Callaway & Mahall, 2007). Plants may allocate resources differently depending on the genetic relatedness of neighbors, and kin recognition should therefore increase fitness (Chen et al., 2012). Cooperative behavior towards close relatives is often seen in a dense population caused by limited dispersal (Cheplick & Kane, 2004; Murphy & Dudley, 2009; Platt & Bever, 2009). Despite the propensity for long-distance dispersal (recorded at up to 250 m) in *M. polymorpha* (Drake, 1992), the current study demonstrated that late-successional *glaberrima*, which naturally dominates mesic and wet forests on Hawaii Island, can recognize and/or respond differentially to sibling neighbors. Evidence for this same ability was weak or absent in early-successional *incana*, the colonizer of new lava flows. The changes in seedling growth in response to neighboring genetic relatedness in *glaberrima* suggests that this variety has the capacity to recognize, and/or the capacity to differentially respond to, the genetic relatedness of neighboring plants. Since the signals between seedlings were not examined in this study, it is impossible to distinguish between the roles of recognition and response in the observed variation in growth of target seedlings among the four treatments.



The greater potential for fine-scale genetic structure in high-density late-successional forests relative to the low-density populations on new lava flows may have promoted the greater ability to recognize and/or respond to kin in *glaberrima* relative to *incana*. Although the dispersibility of *M. polymorpha* seeds by wind is high (Drake, 1992), a significant fraction of seeds is likely to fail to disperse far beyond the maternal tree in (Alvarez-Buylla & Martínez-Ramos, 1990; Drake, 1998). Restricted seed dispersal is likely more common in the denser canopies of late-successional forests than in sparsely-population lava fields. In addition, given that the germination rate was drastically reduced when *Metrosideros* seeds were slightly buried from the surface (< 1% at 5 mm deep; < 45% at 2 mm deep; 90% germination rate at the surface) as well as in the dark condition (< 10%; > 90% with indirect sunlight) (Drake, 1993), *incana* seeds may have low germination rate on the uneven lava rock with cracks. Together, the differences in seed dispersal and germination rates anticipated between early- and late-successional environments on Hawaii Island may lead to relatively finer genetic structure within populations of *glaberrima*.

The relationship between plants (cooperation or competition) can be affected by genetic relatedness of neighboring plants and the outcome of cooperation or competition can appear as a change in resource allocation in plants (Murphy & Dudley, 2009). The vertical growth of plants both above- and belowground can increase resource acquisition, while the horizontal growth may cause competition by overlapping with neighboring plants. In this study, increased allocation to root length versus shoot length with sibling seedlings was observed in *glaberrima* while a slight decrease in root:shoot length ratio with siblings was shown in *incana*. Since shoot length was consistent among the treatments, the variation of root:shoot length ratio was predominantly due to variation in root length, suggesting the importance of roots to the recognition and/or response

to the genetic relatedness of neighbors. Given that the root:shoot mass ratio was less than one in all treatments, seedlings allocated slightly more to shoots than to roots in terms of mass. Since there is no difference in root:shoot mass ratio among treatments, as was true for shoot length, the genetic relatedness of neighboring seedlings did not affect root mass, shoot mass, or shoot length in *M. polymorpha*, but affected root length. In *glaberrima*, the increased allocation to root length with sibling neighbors while maintaining relatively low root mass appears consistent with a previous study that showed increased root length and decreased root radius with conspecific neighbors in both grass and forb species (Jastrow & Miller, 1993). When growing alongside closely related neighbors, *glaberrima* siblings may search for nutrients by increasing root length instead of mass, thereby minimizing competition with neighbors.

In addition to the cooperative behavior observed belowground with close relatives in *glaberrima*, seedlings of this variety appeared to avoid competition with siblings aboveground as well. SLA is a reliable predictor of a plant's tolerance to a competitor (= competitive response) because it reflects the ability to change light-absorption area with a unit leaf mass, and light availability varies with competition (Cyrille Violle et al., 2009). In this study, the highest SLA in both *incana* and *glaberrima* was observed in the sibling treatment in the absence of mycorrhizal fungi, yet the effect of the mycorrhizae  $\times$  treatment interaction term on SLA was significant only in *glaberrima*. Given that shoot length was consistent among treatments, *glaberrima* increased light-capture efficiency with sibling neighbors by increasing SLA without increasing shoot height, suggesting cooperation with siblings through alteration of above-ground growth in *glaberrima*. Since the change in light-capture efficiency was only observed in the absence of mycorrhizal fungi, the increase in SLA indicating cooperative behavior between sibling plants may appear only under limited nutrient conditions. This finding is consistent with a previous

study of *Trifolium repens* in which SLA increased with no change in petiole length in plants grown with siblings at high density (= presumably low nutrient availability, Lepik et al., 2012).

Cooperative traits and limiting resources in native environments are associated in some plant species, as shown in the reduced growth aboveground with close relatives in *Impatiens pallida* growing in shady areas (Murphy & Dudley, 2009) and low root growth in proximity with relatives in *Cakile edentula* in sand (Dudley & File, 2007). In natural habitats of *M. polymorpha*, *incana* is limited to soil nutrients on lava rocks while *glaberrima* has low light availability in forests. The improved light-capture efficiency coupled with minimized competition with neighbors that was achieved through increasing SLA but not increasing shoot length may be a cooperative trait in *glaberrima* growing in the presence of siblings.

Finally, this study also revealed a significant negative effect of neighbor seedling size on target seedling size *except* in the sibling treatment for *glaberrima*, which provided further evidence of kin recognition/response in *glaberrima*. The total dry masses of the neighbor and target seedlings were inversely related in almost all treatments for both (target) varieties. The single exception was the sib treatment for *glaberrima* target seedlings in which neighbor- and target-seedling dry masses were positively correlated. Sibling seedlings of *glaberrima* growing in proximity may facilitate each other's growth regardless of the presence of mycorrhizal fungi. It appears that, when growing with close relatives, seedlings of *glaberrima* avoid competition and increase the efficiency of nutrient acquisition by increasing root length with constant root mass and of light capture by increasing SLA without changing shoot length. These modifications may allow the formation of high-density forests on mature substrates at low-to-middle elevations on Hawaii Island in which *glaberrima* forms a mono-dominant canopy. The current study may be the first to suggest evidence for cooperative behavior in tree species through both above- and

below-ground measures. Both lines of evidence suggest that seedlings of late-successional *glaberrima* have the ability to recognize and/or respond differentially to the genetic relatedness of neighboring seedlings, and that this ability is weak or absent in early-successional *incana*.

#### **3.4.4 Lack of evidence that mycorrhizal fungi facilitate kin recognition in both *incana* and *glaberrima***

This study failed to show any impact of mycorrhizae on the recognition or response of seedlings of *M. polymorpha* to the genetic relatedness of neighboring seedlings. Given that plants can perceive the environment through microbes such as mycorrhizal fungi in addition to plant sensory organs, including leaves and roots (Biedrzycki et al., 2010; Richard Karban et al., 2014; Van Der Heijden et al., 2015), mycorrhizal fungi may not only promote plant growth but may also facilitate kin recognition (File, Klironomos, et al., 2012). In this study of two successional varieties of *M. polymorpha*, however, seedlings behaved differently towards genetically different neighbors, regardless of the presence of mycorrhizal fungi. In addition, the increased efficiency of resource acquisition with siblings shown as high allocation to root length and high SLA in the sib treatment was demonstrated regardless of the presence of mycorrhizal fungi and in the absence of mycorrhizal fungi, respectively. Lastly, the positive relationship between the biomasses of the target and neighboring seedlings in the *glaberrima* sib treatment was observed both with and without mycorrhizal symbionts. Overall, mycorrhizal fungi increased seedling growth but did not appear to facilitate kin recognition in *M. polymorpha*.

The absence of any impact of mycorrhizal fungi on interactions between neighboring seedlings indicates that kin recognition and/or response in *M. polymorpha* occurs without the aid of symbionts. Based on the current study design, in which seedlings were free to communicate

aboveground, communication between neighbors through volatile molecules aboveground cannot be ruled out. Nonetheless, communication through belowground interactions between roots, probably through root exudates, appears to be more likely.

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## **4. Variation in mycorrhizal growth in response to the genetic relatedness of neighboring seedlings of *M. polymorpha***

### **4.1 Introduction**

Mycorrhizal fungi can form complex networks underground that connect neighboring plants and play an important role in structuring ecosystems. A majority of plant species form mutualistic relationships with mycorrhizal fungi, given the benefits of this relationship to both partners (M. C. Brundrett, 2009). In general, mycorrhizae promote nutrient uptake and resistance to biotic and abiotic stress in plants, while plants provide carbohydrates to the mycorrhizae (Gorzalak et al., 2015; S. E. Smith & Read, 2008). Since mycorrhizal symbionts can affect host-plant growth and survival (Simard, 2018), the potential for mycorrhizae to shape plant communities is considerable (0-57 %), through their effects on competition and herbivory (Klironomos et al., 2011). Although our understanding of how mycorrhizas affect host plants is growing (Babikova et al., 2014; van der Heijden & Horton, 2009), how host plants influence mycorrhizal symbionts is still poorly understood.

A growing body of studies suggests that the abundance of mycorrhizal fungi in soil can be used to distinguish between cooperative and competitive interactions between neighboring host plants (Engelmoer & Kiers, 2015; File, Klironomos, et al., 2012). The carbon required for the growth and survival of mycorrhizal symbionts is supplied solely by host plants through photosynthesis to arbuscular mycorrhizal (AM) and some ectomycorrhizal (ECM) fungi (Bogar et al., 2022; Engelmoer & Kiers, 2015). At least 10% of photosynthates is allocated to mycorrhizal symbionts (Ji & Bever, 2016), and this value may reach as high as ~20%, depending on the amount of phosphorus mycorrhizal symbionts provide to the host plants, the resource availability of the local environment (Christian & Bever, 2018), and the nature of the relationship

between host plants (File, Klironomos, et al., 2012). Greater mycorrhizal length was observed in pots with closely related host plants (i.e., siblings) compared to pots with distantly related, conspecific neighbors (File, Klironomos, et al., 2012) and neighbors of different species (Engelmoer & Kiers, 2015). Because allocation of photosynthate to mycorrhizae is costly to host plants, the greater mycorrhizal growth in sibling pots, which necessitated increased carbon allocation from the host plants, was interpreted as evidence of cooperative behavior between sibling neighbors (File, Klironomos, et al., 2012). Not all studies of this type, however, have shown higher mycorrhizal growth in pots with closely related host plants relative to pots with distantly related host plants (Derelle et al., 2012), and no such studies have been carried out on trees.

The density of mycorrhizal fungi in soil varies with both nutrient availability and accessibility to host plants (Dickie et al., 2013), and as such is expected to differ between early- and late-successional substrates. In general, early successional sites are characterized by an abundance of primarily rock-derived phosphorus and limited availability of soil nitrogen for plant growth (Dickie et al., 2013). Through subsequent ecosystem development, the shift of nutrient availability occurs from a state of rich phosphorus/limited nitrogen to a state of high nitrogen availability through biological fixation, to an eventual state of dual phosphorus-and-nitrogen limitation (Dickie et al., 2013). At early successional stages, where competition in plant communities is weak, interactions between host plants and mycorrhizal symbionts are expected to be mutually positive, leading to increased local density of both partners (Dickie et al., 2002b, 2013; Nara, 2006a). In the more dense communities of later successional stages, in contrast, stronger competition between plants should lead to negative interactions between hosts and

mycorrhizal fungi, promoting species replacement, and ultimately, coexistence (Bever, 2002; Bever et al., 1997).

On the youngest of the main Hawaiian Islands, Hawai`i Island, plant communities occur on a mosaic of substrates of various ages associated with ongoing volcanic activity. The different successional stages on Hawai`i Island support different varieties of the landscape-dominant tree, *Metrosideros polymorpha* (Myrtaceae): the pubescent, early-successional variety *incana* (hereafter *incana*) and the glabrous, late-successional variety *glaberrima* (hereafter *glaberrima*). The pioneer *incana* establishes on at least four-year old lava flows below ~1,200 m above sea level, while *glaberrima* dominates mature, wet and mesic forests on late-successional substrates (Drake & Mueller-Dombois, 1993; Kitayama et al., 1995; Smathers & Mueller-Dombois, 1974). In addition to their contrasting responses to light (Zangaro et al., 2000b), *incana* and *glaberrima* occur in highly contrasting population densities, mycorrhizal abundances, and soil nutrient availabilities, all of which are higher for late-successional *glaberrima* (Kitayama & Mueller-Dombois, 1995; Koske et al., 1992).

Nearly all native Hawaiian plant species in forests, coastal shrublands, and even recent volcanic substrates form associations with AM fungi (Koko et al., 2021; Koske et al., 1992). In fact, the frequency of plant-AM fungal associations in Hawaii's native flora (90%) (Koko et al., 2021) may be greater than the estimated average for plants globally (~82%) (M. C. Brundrett, 2009). Given that the AM colonization rate is often high at early-successional stages (Zangaro et al., 2003), it may be that AM fungi may play a significant role in supporting plants on the young substrates of Hawai`i Island. Further, because mycorrhizal abundance increases with successional stage in Hawai`i (Koske et al., 1992), and elsewhere (Lambers, Raven, et al., 2008), the responsiveness of plants to mycorrhizal fungi may be expected to differ between early- and



late-successional Hawaiian plants (Middleton & Bever, 2012; Zangaro et al., 2003). The low density of incana populations and low mycorrhizal abundance characteristic of early-successional sites are expected to limit encounters between incana and mycorrhizal symbionts. On the other hand, the high population densities and high mycorrhizal abundances characteristic of late-successional substrates should promote interactions between glaberrima and mycorrhizae. Furthermore, responsiveness of plants to mycorrhizae is expected to decrease with succession (Zangaro et al., 2000b), a prediction that was supported by the higher mycorrhizal responsiveness in incana than glaberrima observed in the current study (Chapter 3).

Examining mycorrhizal development with differently related host plants is not only helpful for understanding how the genetic relatedness of neighboring host plants affects mycorrhizal growth but also for distinguishing between cooperative and competitive behavior of the host plants. Quantifying mycorrhizal development can help to determine plant behavior under the premise that cooperative neighboring plants invest more to mycorrhizal growth than do competing neighbors. In this study, mycorrhizal fungal length in the soil was estimated as a measure of mycorrhizal growth in replicate pots comprising focal seedlings of either incana or glaberrima and neighbors of varying degrees of genetic relatedness to the focal seedlings. I predicted that mycorrhizal length would vary in response to both the successional stage of the focal plants and the relatedness of neighboring seedlings within pots.

## **4.2 Materials and methods**

### **4.2.1 Experimental setting**

Mycorrhizal fungal length was quantified in the soil of pots used in an experiment designed to reveal plant-plant interactions between neighboring seedlings that varied in genetic

relatedness, described in Chapter 3. Briefly, after germination, seedlings (~ 0.5 cm tall) were transferred in pairs to individual 5 x 5.5-cm pots, each containing one target seedling and one neighbor seedling. The target seedlings were split evenly between *incana* and *glaberrima* and were allocated among four treatments according to the genetic relatedness of the neighbor to the target: alone (see below), sibling (sib), pop (neighbor derives from a different population of the same variety), and var (neighbor is of the opposite variety). For the “alone” treatment, the soil in the pot was divided evenly in half with a plastic divider. This was done to ensure roughly uniform soil availability across target seedlings regardless of treatment. Further, one half of the pots were grown in mycorrhizae-free media, while the other half were grown in media supplemented with a mix of AM and ECM fungi (see Chapter 3 for details). The seedlings were grown for ~15 months in a greenhouse (approximately 800  $\mu\text{mol}/\text{m}^2/\text{s}$ ) with overhead water three times per day for five minutes at the University of Hawai‘i Hilo’s Pana‘ewa Farm at ~100 m in elevation. After 15 months of growth, seedlings were harvested, and soil hyphal length was examined from the pots with and without mycorrhizal fungi.

#### **4.2.2 Estimation of mycorrhizal fungal length in soil**

The length of mycorrhizal fungi in experimental pots was estimated following the method for estimating hyphal length by M. Brundrett et al. (1994) with modifications. Briefly, for each pot examined, soil cores (soil profiles) were collected from four positions using a reagent digger and pooled in a single sample. After the cinder and rocks were removed, a small amount of water was added to homogenize the sample. Approximately 2.0 g of the sample was then transferred into a 500-mL beaker, and the exact wet weight was recorded. To this, 100-ml deionized water and 12-ml Calgon solution (35.7 g/L sodium hexametaphosphate) were added,

and the solution was allowed to settle for 30 min. Subsequently, the soil-mix solution was vigorously stirred for one minute using a glass rod to break up aggregates. The solution was further stirred on a magnetic stirrer to allow large particles and aliquots to separate. After 10-ml aliquots were transferred to a new 200-mL beaker, 50 ml of deionized water and 6 ml of Calgon solution were added. After 30 seconds, the soil mix was vigorously stirred again, and 10 ml of the solution was filtered through 20-um nylon mesh and collected into a falcon tube. After 5-ml vinegar and 0.25-ml blue ink were added into each falcon tube, the solution was vortexed for 30 seconds and stained for 1.5 hours. This simple and safe ink-and-vinegar staining technique has been shown to be as effective as the traditional trypan-blue stain for hyphae (Vierheilig et al., 1998). The stained solution was filtered through a cellulose nitrate filter paper (Whatman 47-mm diameter, 0.45- $\mu$ m pore size) to collect the blue-stained hyphae.

The filter paper was cut into quarters to facilitate mounting on microscope slides. Each slide with a  $\frac{1}{4}$ -sized filter paper was viewed at 200X magnification under a light microscope (Leica DM 500, Leica Camera AG, Wetzlar, Germany), and images were taken through the optical scope with a digital camera (Canon PowerShot SD 980 IS, Canon Inc., Tokyo, Japan). From each soil sample, 50 digital images were haphazardly obtained to sample broadly across each of the  $\frac{1}{4}$ -cut filter papers, and the step was repeated with a second  $\frac{1}{4}$ -cut filter paper for each sample, so that one-half of the filter paper was scanned. A grid layer formed by 12 horizontal and 9 vertical lines intercrossed perpendicularly was placed onto each image using PowerPoint slides (Microsoft PowerPoint 2010). The number of positions where the hyphae crossed a vertical or horizontal gridline was counted in all 50 images along each of the two  $\frac{1}{4}$ -sized filters as the hyphal count (100 images from  $\frac{1}{2}$ -sized filters total per sample). Any blue

line that was between 1.0-13.4  $\mu\text{m}$  in diameter was scored as a mycorrhizal hypha (Boddington et al., 1999). The hyphal counts were summed across the two  $\frac{1}{4}$ -filter papers per sample.

Total hyphal length (mm) from the two  $\frac{1}{4}$ -sized filters was quantified using the equation modified from the Tennant equation that originally used to estimate root length of cotton and wheat (Shen et al., 2016; Tennant, 1975).

$$L = \frac{C \times \left(\frac{11}{14}\right) \times g \times A_f}{A_g \times N_i}$$

where,

$\frac{11}{14}$  is a constant

$C$  is the total hyphal counts in 100 images

$A_f$  is the filter paper area ( $= (23.5^2 \times \pi) / 2 = 867.4 \text{ mm}^2$ )

$A_g$  is the area of grid net ( $= 0.05 \times 0.05 \times 12 \times 9 = 0.27 \text{ mm}^2$ )

$N_i$  is the number of images ( $= 50 \times 2 = 100$ )

$g$  is the grid unit ( $= 0.05 \text{ mm}$ )

Soil hyphal length was calculated as follows:

$$\text{Soil hyphal length (m/g soil)} = \text{hyphal length (mm)} / (1000 \times \frac{1}{2} \text{ of the soil weight, g})$$

Since hyphal lengths were quantified from  $\frac{1}{2}$ -sized filters per sample,  $\frac{1}{2}$  of the soil weight (g) was used to calculate soil hyphal length.

### 4.2.3 Statistical analyses

Soil hyphal length was analyzed using ANOVA to examine the effect of treatment and variety as well as the interaction between treatment and variety using R ver.4.0.5 (R Development Core Team, 2001). Additional ANOVA were performed for target seedlings of *incana* and *glaberrima* separately to identify any taxon-specific effects of treatment on soil hyphal length. Finally, because hyphal length might be influenced by plant size, Pearson correlation coefficients ( $r$ ) were calculated between soil hyphal length and each of total dry mass, root mass, and root length of the focal seedling.

## 4.3 Results

### 4.3.1 Greater mycorrhizal length for *incana* than *glaberrima*

The effect of the interaction between treatment and variety on mycorrhizal length was not statistically significant ( $\chi^2 = 1.19$ ,  $df = 3$ ,  $p = 0.756$ ). Mycorrhizal fungal length was significantly greater in pots with *incana* target seedlings than in those with *glaberrima* target seedlings ( $\chi^2 = 5.25$ ,  $df = 1$ ,  $p = 0.022$ , Fig. 4.1). Although total dry mass of the target seedlings was also greater for *incana* than for *glaberrima*, there was no relationship between mycorrhizal length and total dry mass of the target seedling ( $r = -1.03$ ,  $df = 180$ ,  $p = 0.304$ , Fig. 4.2). In addition, no correlation was found between mycorrhizal length and root mass of the target seedling ( $r = -0.08$ ,  $df = 179$ ,  $p = 0.262$ ) or root length ( $r = 0.04$ ,  $df = 180$ ,  $p = 0.612$ ).

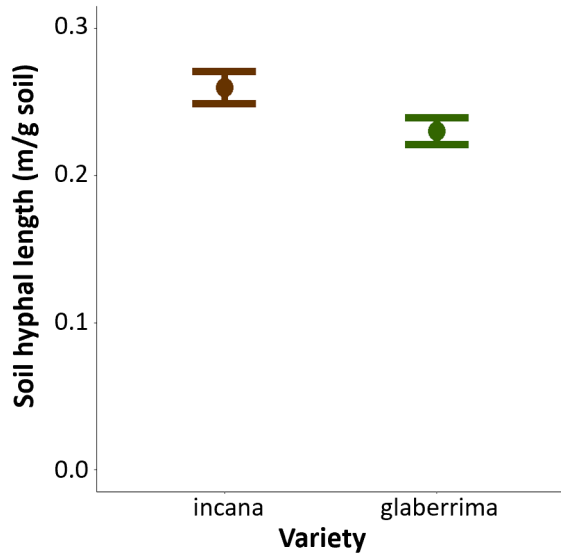


Fig. 4.1 Mean  $\pm$  SE soil hyphal length measured after  $\sim$ 15 months in experimental pots with target seedlings of *M. polymorpha* var. *incana* or var. *glaberrima*.

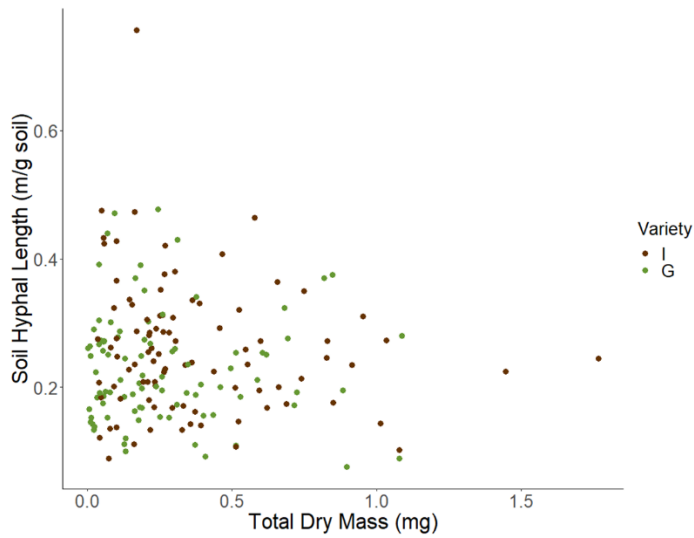


Fig. 4.2 Relationship between the total hyphal length in the soil and total dry mass of the target seedling after  $\sim$ 15 months of the experiment with mycorrhizal fungi. Brown dot: *incana*, green dot: *glaberrima*.

### 4.3.2 Increased mycorrhizal length in pots with sibling neighbors

The soil hyphal length was significantly affected by treatment ( $\chi^2 = 10.75$ ,  $df = 3$ ,  $p = 0.013$ ). The mycorrhizal hyphae were longer in the sib treatment compared to other treatments (Fig. 4.3). When the two varieties of *M. polymorpha* were analyzed separately, the significant effect of treatment on soil hyphal length was found in glaberrima ( $\chi^2 = 10.48$ ,  $df = 3$ ,  $p = 0.015$ ), but not in incana ( $\chi^2 = 2.50$ ,  $df = 3$ ,  $p = 0.476$ ).

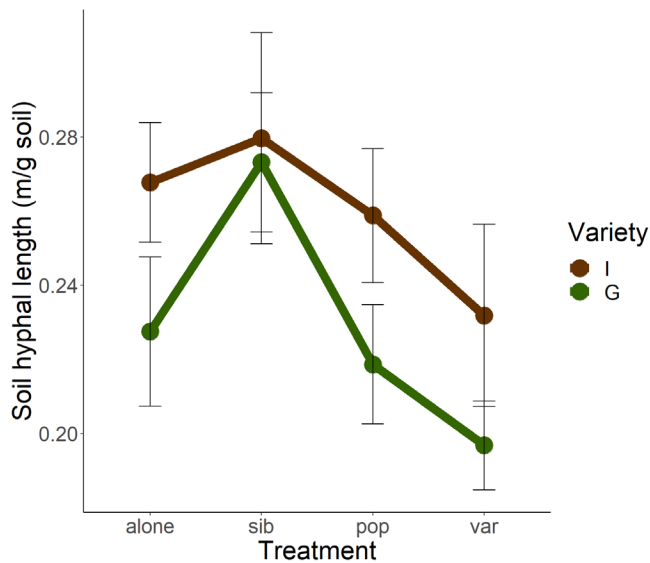


Fig. 4.3 Mean  $\pm$  SE soil hyphal length estimated at the end of the 15-month experiment with four treatments (alone, sib, pop, and var). Brown: incana, green: glaberrima.

For glaberrima only, soil hyphal length and root length of the target seedling showed similar patterns across treatments, with peak growth in the sib treatment (Fig. 4.4). Although hyphal length and root length were not directly correlated ( $t = 0.51$ ,  $df = 180$ ,  $p = 0.612$ ), the

effects of treatment on both soil hyphal length (see above) and root length (see Chapter 3) were statistically supported.

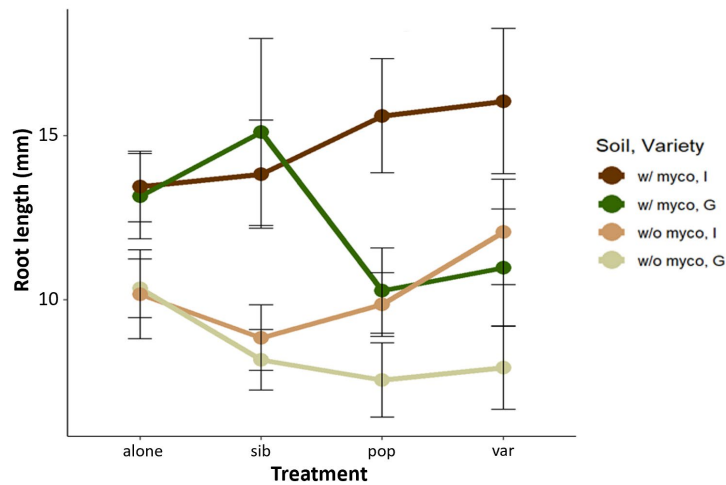


Fig. 4.4 Root length of seedlings in *Metrosideros polymorpha* var. *incana* (brown) and var. *glaberrima* (green) with (darker) and without mycorrhizal fungi (lighter) grown alone, with a sibling, with a different population within the same variety, or with the opposite variety for ~15 months in a greenhouse.

#### 4.4 Discussion

##### 4.4.1 Difference in mycorrhizal length between early- and late-successional varieties of *M. polymorpha*

This is the first study to demonstrate differences in mycorrhizal growth between host plants that specialize in early- and late-successional stages. Mycorrhizal growth depends on the flow of carbohydrates produced by host plants through photosynthesis. Since nutrient



availability in soils, mycorrhizal abundance, and mycorrhizal dependency in plants can vary along successional gradients (Dickie et al., 2013; Kitayama & Mueller-Dombois, 1995; Koske et al., 1992), the strategies of nutrient allocation may be expected to differ between early- and late-successional plants (Cordell et al., 2001), thus affecting mycorrhizal growth.

Mycorrhizal length was affected differently by the two successional varieties of *M. polymorpha* at the early stages of the seedling development. Greater total hyphal length in the soil was observed in pots with incana target seedlings relative to pots with glaberrima target seedlings. Plants usually allocate an average of 10-20 % of photosynthetic products to mycorrhizal fungi (Dickie et al., 2013). The results of the current study imply that relative to seedlings of glaberrima, seedlings of incana donated more carbon to mycorrhizal fungi. Facilitation of mycorrhizal length in early-successional incana is consistent with positive feedback between host plants and mycorrhizal symbionts, a feature commonly seen in the earlier stages of succession (Dickie et al., 2002a, 2013). Mycorrhizal fungi are expected to be sparse in early-successional substrates. Through the allocation of carbon to mycorrhizal fungi, plants established at such sites may increase local mycorrhizal density and benefit from increased nutrient uptake through a common mycelium network (Dickie et al., 2002c; Nara, 2006b). On the other hand, high growth rates of mycorrhizal fungi at late-successional sites would likely promote growth, and thus intensified competition, among host plants. The negative interactions between plants and mycorrhizal fungi that are expected at late-successional sites (Dickie et al., 2002a, 2013) are consistent with the apparent lower carbohydrate allocation by glaberrima to mycorrhizal fungi in this study.

Given prior evidence of AM associations with *M. polymorpha* (Koske et al., 1992), it may be that the greater hyphal length in pots with incana target seedlings was due to a positive

interaction between *incana* and AM fungi rather than with ECM fungi. In natural habitats, however, it is possible that *M. polymorpha* is associated with ECM fungi. ECM fungi occur in Hawaiian Island substrates (Koske et al., 1992), and dual AM and ECM infection appear to be common in early-successional sites (Dickie et al., 2013). The ECM fungus used in the current study (*Pisolithus tinctorius*) may have a positive interaction with *incana* by aiding mainly in nitrogen uptake (Read, 1991).

In spite of the greater seedling growth (e.g., total dry mass) and mycorrhizal length observed for *incana* relative to *glaberrima*, no significant correlation was found between these measures. This suggested that the greater mycorrhizal length observed in the pots with the *incana* target seedlings was not simply due to the generally larger size of *incana* seedlings. The lack of relationship between plant size and mycorrhizal length is consistent with findings of earlier studies that showed no difference in aboveground plant biomass among treatments (sibling host plants vs. strangers) and greater soil hyphae length in experimental pots with siblings compared to pots with strangers.

The lack of a correlation between seedling size and mycorrhizal length is consistent with other results of this study that suggest a positive interaction between *incana* seedlings and mycorrhizal symbionts and a less positive (or negative) interaction between *glaberrima* and mycorrhizae. Since seedling size (i.e., total dry mass) was greater in *incana* than *glaberrima* regardless of the presence of mycorrhizal fungi (see Chapter 3), higher seedling growth rate appears to be a characteristic of *incana* as an early-successional plant (Grime, 1977; Koziol et al., 2015). Given that the responsiveness to mycorrhizal fungi was greater for *incana* than *glaberrima* (i.e., mycorrhizal fungi promoted *incana* growth more than *glaberrima* growth; Chapter 3), and that mycorrhizal length was higher in *incana* pots compared to *glaberrima* pots, it

would be safe to say that *incana* and mycorrhizal fungi have a positive interaction. On the other hand, lower mycorrhizal responsiveness and less investment to mycorrhizal symbionts in *glaberrima* compared to *incana* suggest less positive (or negative) interactions between *glaberrima* and mycorrhizal fungi. The pattern of interaction between *M. polymorpha* and mycorrhizal symbionts at the early- and late-successional stages is consistent with the pattern commonly seen along successional gradients (Bever, 2002; Dickie et al., 2013).

#### **4.4.2 Increased mycorrhizal length with sibling seedlings of *M. polymorpha***

Although the influence of the genetic relatedness of neighboring host plants on local mycorrhizal growth is gaining attention, studies of this relationship have been restricted to herbaceous plants and have produced mixed results (Derelle et al., 2012; Engelmoer & Kiers, 2015; File, Klironomos, et al., 2012; Ronsheim & Anderson, 2001). As such, the effect of relatedness of neighboring host plants on mycorrhizal development is still not well understood, especially in trees. Results to date show that closely related host plants can have a positive or no influence on local mycorrhizal development (Derelle et al., 2012; Engelmoer & Kiers, 2015; File, Klironomos, et al., 2012). Given the possible importance of genetic relatedness on mycorrhizal development and the ecological differences between early- and late-successional varieties of *M. polymorpha*, I expected that mycorrhizal length in the soil would vary according to the genetic relatedness of neighboring host seedlings and that the intensity of mycorrhizal length would differ between *incana* and *glaberrima* hosts.

In this study, the greatest mycorrhizal length was observed when sibling seedlings were grown together compared to other treatments (i.e., grown alone or with distantly related neighbors) suggesting that more carbon may flow from neighboring seedlings to mycorrhizal

fungi when they are siblings. Since sibling seedlings of *M. polymorpha* grown together appear to promote mycorrhizal length through relatively greater allocation of carbon to mycorrhizas, they appear to fit a model of cooperation rather than competition.

The evidence for cooperation between neighboring siblings seems to be stronger for late-successional glaberrima than early-successional incana. Although both incana and glaberrima showed peak mycorrhizal length with sibling seedlings, the change in mycorrhizal length in response to the relatedness of *M. polymorpha* seedlings was statistically supported only in glaberrima. This result implies that glaberrima siblings allocated a significant amount of carbon to mycorrhizae in a form of cooperation, which is consistent with the earlier work showing greater mycorrhizal length in experimental pots with siblings of *Ambrosia artemisiifolia* L. relative to pots with strangers (File, Klironomos, et al., 2012). Based on the study of the growth of *M. polymorpha* seedlings in response to neighbors of varying genetic relatedness (Chapter 3), glaberrima siblings seem to avoid overlap (competition) and increase the efficiency of nutrition acquisition by increasing root length without expanding root mass as well as enhance light-capture capacity by increasing specific leaf area without increasing the shoot height. For glaberrima, which has a high likelihood of interacting with other plants in its native high-density, late-successional forests, the ability to recognize and/or respond to the relatedness of neighboring plants would likely be favored by selection. Given the importance of mycorrhizal networks as conduits for the exchange of water, nutrients, and signals between plants (Lanfranco et al., 2016; Simard, 2018; W. Wang et al., 2017), mycorrhizas should play a more important role in plant-plant interactions in glaberrima than in incana, affecting the growth of both seedlings and mycorrhizas.

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## **5. Conclusions and future directions**

### **5.1 Mycorrhizal fungi increase growth rate of *M. polymorpha* but do not appear to facilitate kin recognition and response**

Mycorrhizal fungi promoted growth in two successional varieties of *M. polymorpha*, *incana* and *glaberrima*, but there was no sign of mycorrhizal symbionts facilitating kin recognition in *M. polymorpha*. All seven measures of seedling growth showed a positive influence of mycorrhizal symbionts on *M. polymorpha* growth. Furthermore, mycorrhizal fungi are known to aid nutrient uptake, and *M. polymorpha* seedlings allocated less to root length than shoot length in the presence of mycorrhizal symbionts, while seedlings without symbionts increased allocation to root length. These results suggest that this species is highly responsive to mycorrhizal fungi.

The ability to recognize and/or respond to genetically different neighbors was observed in *M. polymorpha* both with and without mycorrhizal symbionts, suggesting that mycorrhizal fungi do not facilitate kin recognition in this species. The increased SLA in the sibling treatment in the absence of mycorrhizae and the positive correlation between neighboring siblings in terms of total dry mass both with and without mycorrhizal fungi also support the idea that *M. polymorpha* can recognize and/or respond to neighboring siblings without the aid of mycorrhizal networks.

### **5.2 Higher growth rate and investment in mycorrhizal length in early-successional *incana* relative to late-successional *glaberrima***

Comparison of the two varieties revealed that, relative to seedlings of *glaberrima*, those of *incana* achieved a larger size and supported greater mycorrhizal length. Almost all measures

of growth (with one nearly significant difference in root dry mass) were greater for *incana* than for *glaberrima*. Combined with the lack of correlation between total dry mass and mycorrhizal length, these findings are consistent with faster growth of early-successional *incana* relative to late-successional *glaberrima* at early seedling stages regardless of the presence of mycorrhizal fungi. Rapid seedling growth is likely adaptive on young lava flows, where conditions favorable for seedling establishment are unpredictable and short-lived.

In addition to the difference in seedling size between *incana* and *glaberrima*, the interaction between *M. polymorpha* seedlings and mycorrhizal fungi seems to range from a positive interaction in *incana* to a negative interaction in *glaberrima*. Considering that early-successional *incana* occurs in an environment with low population density as well as low mycorrhizal abundance, *incana* is expected to interact with mycorrhizal fungi less frequently. When *incana* and mycorrhizal symbionts do come into contact, both organisms may facilitate the growth of each other without intensifying competition, leading to positive feedback. On the other hand, the late-successional *glaberrima*, which interacts frequently with other plants and mycorrhizal symbionts in dense, late-successional environments, may limit their own growth as well as investment in mycorrhizae in order to minimize competition. This negative feedback is commonly seen in the later stages of succession and promotes species coexistence.

### **5.3 Stronger recognition of/response to genetically different neighbors in late-successional *glaberrima***

This study produced four lines of evidence that suggest that seedlings of *glaberrima* are adapted to recognize and/or respond differentially to sibling neighbors compared to more

distantly related neighbors, 1) higher allocation to root length than shoot length with siblings, 2) greater mycorrhizal length with siblings, 3) greater SLA in the absence of mycorrhizal fungi with siblings, and 4) a positive correlation between the growth rates (biomasses) of neighboring sibling seedlings. These findings for *glaberrima* were not observed in *incana* and are consistent with the contrasting population densities of these taxa in nature. Individuals of *glaberrima* are more likely to interact with other *glaberrima* in late-successional wet and mesic forests, where seed dispersal is likely restricted and the ability to recognize and/or respond to the genetic relatedness of neighbors may increase indirect fitness. In these high-density populations, *glaberrima* may have evolved to allocate resources in a way that minimizes competition with close relatives, as was seen for example in the increased root length which minimizes overlap with neighbors. The higher allocation of carbon by *glaberrima* siblings to mycorrhizal symbionts was strongly suggestive of cooperative behavior.

This research provides novel insights into the poorly known world of neighboring interactions in trees. The findings on the effects of neighbor genetic relatedness, especially the “sibling effect,” on two successional varieties in *M. polymorpha* aid understanding of plant-plant interactions within Hawaii’s landscape-dominant tree. *M. polymorpha* is currently suffering from an invasive fungal pathogen with an annual mortality rate of ~10% (Cannon et al., 2022; Hughes et al., 2021). The results of this research may be applied to forest restoration practices in Hawai`i and elsewhere.

## 5.4 Future directions

This study highlights the ability of *glaberrima* to recognize and/or respond to close relatives and cooperate with them. To further understand kin selection in this tree, this study should be repeated with siblings as well as unrelated individuals from the same population. If kin selection favors traits that help relatives, it should also favor traits that avoid helping non-relatives (Lyon, 2003). By comparing maternal half-siblings used in this study (coefficient of relatedness,  $r = 1/4$ ) and unrelated seedlings ( $r \approx 0$ ), observations of seedling behavior in *M. polymorpha* would improve our understanding of plant-plant interactions in *M. polymorpha*.

Plants produce root exudates to communicate with neighboring plants as well as with mycorrhizal symbionts (Bais et al., 2006). Previous work on metabolomics and proteomics suggested that *Arabidopsis thaliana* can release different levels of secondary metabolites and defense-and stress-related proteins into the soil depending on neighboring genetic relatedness (Badri et al., 2012). Identification and quantification of root-secreted secondary metabolites and proteins in *M. polymorpha* would give us valuable insight into the mechanisms underlying plant-plant interactions in this species.

Recent studies are paying more attention to the significance of hormones such as strigolactones that can affect plant growth and symbiotic relationships (Bedini et al., 2018; Chanclud & Morel, 2016; Landgraf et al., 2012). Strigolactones are known to affect AM hyphal branching as well as the germination of root-parasitic plants such as *Phelipanche ramosa* (Soliman et al., 2022; Steinkellner et al., 2007). Although strigolactones do not always affect hyphal branching activity on AM fungi, especially from AM non-host plants (Steinkellner et al., 2007), germination of parasitic plants induced by root exudates of *M. polymorpha* may reveal the different level of strigolactones produced by closely and distantly related *M. polymorpha*.

Further work to identify the genes responsible for plant interactions in *M. polymorpha* would expand our knowledge on plant-plant interactions. It would be helpful to understand what kind of hormones plants produce in the presence of genetically different neighbors and how the plant hormones affect plant growth and mycorrhizal associations and growth, especially in the presence of close relatives. RNA-seq analysis could reveal differentially expressed transcripts related to the biosynthesis of strigolactones (Banasiak et al., 2020; Kretzschmar et al., 2012; Mashiguchi et al., 2021).

Although the importance of mycorrhizal fungi to kin recognition and/or response was not demonstrated in this study, mycorrhizal fungi affected seedling growth in both *incana* and *glaberrima*. In addition, mycorrhizal symbionts may also affect seedling establishment (van der Heijden & Horton, 2009). Since a mix of mycorrhizal fungal species were used for the experiment, it is unclear which fungal species affected *M. polymorpha* more strongly. Future work to understand which fungal species interact positively or negatively with *incana* and *glaberrima* may yield insights useful for the restoration of Hawaiian forests.

## 5.5 Literature cited

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## Curriculum Vitae

**Tomoko Sakishima**  
tomokosakishima3@gmail.com

### EDUCATION

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- 2016 – 2024    **Ph.D. in Biological Sciences**  
University of Nevada, Las Vegas, NV                                  Cumulative GPA: 3.90/4.00  
Advisor: Dr. Elizabeth Stacy
- 2012 – 2015    **M.S. in Tropical Conservation Biology and Environmental Science,**  
University of Hawai‘i at Hilo, Hilo, HI.                              Cumulative GPA: 3.87/4.00  
Advisor: Dr. Elizabeth Stacy
- 2006 – 2009    **B.A. in Biology, Minor in Marine Science,**  
University of Hawai‘i at Hilo, Hilo, HI.                              Cumulative GPA: 3.26/4.00  
Dean’s list (Spring 2007/ Spring 2008)
- 2001 – 2005    **Bachelor of Policy Studies,**  
Kwansei Gakuin University, Hyogo, Japan

### EXPERIENCE

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- 2018 – 2020    **Research Assistant,** University of Nevada, Las Vegas, NV
- Assisted with cross-pollinations, seed collection and record-keeping in a long-term study of reproductive isolating barriers within an adaptive radiation of Hawaiian trees
- 2017 – 2018    **Teaching Assistant,** University of Nevada, Las Vegas, NV
- Taught two lab sections of BIOL197 Principles of Modern Biology II per semester
- 2017 – 2018    **Graduate Student Mentor for Southern Nevada Northern Arizona Louis Stokes Alliance for Minority Participation,** University of Nevada, Las Vegas
- Mentored 13 undergraduate students in STEM fields
  - Checked academic performance and supported to plan until graduation
  - Assisted with student applications to summer internship
- 2016 – 2017    **Research Assistant,** University of Nevada, Las Vegas, NV
- Assisted in the set-up of the Stacy lab (molecular biology component) at UNLV
  - Analyzed population genetics data for manuscript preparation
- 2009 – 2016    **Lab Technician,** Research Corporation of the University of Hawai‘i, Hilo, HI

- Assisted in multiple projects on the evolutionary ecology and genetics of Hawaiian plants and insects
- Trained and supported four graduate and five undergraduate students in the genetics lab
- Planned and carried out the Population and Evolutionary Genetics Workshop in the Chemistry Collaborations, Workshops and Communities of Scholars program with Dr. Stacy and Dr. Price

2008 – 2009 **Lab Assistant**, Research Corporation of the University of Hawai'i, Hilo, HI

- Used molecular markers to identify hybrid genotypes in a natural intraspecific hybrid zone of 'ōhi'a *lehua* (*Metrosideros polymorpha*)
- Developed technical skills needed for a broad range of laboratory procedures
- Achieved 100% accuracy in Real-time PCR

2001 – 2005 **Private Tutor**, Trygroup Inc., Osaka, Japan

- Taught biology, math, physics and English to 10- to 15-year-old students individually at their homes; all of these students passed their entrance examinations for high school

## MENTORING ACTIVITIES

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### Graduate students

- Mr. Gary Sur – UH Hilo TCBES M.S.; spring 2016
- Ms. Stephanie Vandruff – UH Hilo TCBES M.S.; spring 2013
- Ms. Tani Wright – UH Hilo TCBES M.S.; spring 2012
- Mr. Nicholas DeBoer – UH Hilo TCBES M.S.; fall 2009

### Undergraduate students

- Ms. Katherine Irelan – Princeton University; Summer 2021
- Ms. Heather Zepeda – UNLV; fall 2020 – fall 2021
- Mr. Abimael Barajas – UNLV; fall 2017 – spring 2018
- Ms. Alyssa Amata – UNLV; fall 2017 – spring 2018
- Mr. Benjamin Azua – UNLV; fall 2017 – spring 2018
- Mr. David Bandbaz – UNLV; fall 2017 – spring 2018
- Ms. Hannah Barbarita – UNLV; fall 2017 – spring 2018
- Ms. Jamie Therese Abad – UNLV; fall 2017 – spring 2018
- Mr. Jizxin Tian – UNLV; fall 2017 – spring 2018
- Ms. Mihaela Genova – UNLV; fall 2017 – spring 2018
- Ms. Samantha Andrews – UNLV; fall 2017 – spring 2018
- Ms. Samantha Lockhart – UNLV; fall 2017 – spring 2018
- Mr. Savva Zoltoev – UNLV; fall 2017 – spring 2018
- Mr. Tam Timothy Phanle – UNLV; fall 2017 – spring 2018

- Ms. Taylor Cassidy – UNLV; fall 2017 – spring 2018
- Mr. Eric Taylor – UH Hilo; fall 2015 – spring 2016
- Ms. Merritt Burch – UH Hilo; fall 2014 – spring 2015
- Ms. Kyung Min Kim – UH Hilo; summer 2012
- Ms. Erin Datlof – UH Hilo; spring 2012
- Ms. Ala Malia Leka – UH Hilo; fall 2010 – fall 2011

### High school student

- Mr. Ethan Okahara-Olsen – Waiakea High School in Hilo, Hawai‘i; fall 2020

### LABORATORY AND ANALYTICAL SKILLS

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DNA extraction • PCR • Real-time PCR • Microsatellite genotyping • Sanger sequencing • AFLP • GBS • Molecular cloning • Growth of competent cells • Agarose gel electrophoresis • Design of microsatellite primers • Pipette calibration • Statistical analysis (R, SPSS, Minitab) • Population genetics software (Arlequin, STRUCTURE, NewHybrids, Genepop, and Splits Tree) • Sequence analysis software (Sequencher, PAST, MEGA, MacClade, FigTree, PAUP, jModelTest, GARLI, MrBayes, and BEAST) • Geographical information system software

### PEER-REVIEWED PUBLICATIONS

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#### In prep

**T. Sakishima**, D. K. Price, A. Cuttriss, and E. A. **Stacy**. Weak evidence of fine-scale local adaptation within the dominant tree, *Metrosideros polymorpha*, along Hawaii Island’s elevation gradient.

Stacy, E. A., **T. Sakishima**, H. Tharp, and N. Snow. (2020) Strong isolation within *Metrosideros* (‘Ohi`a) on O`ahu correlates with extreme environments. Symposium Issue; Journal of Heredity 111: 103-118.

Dupuis, J. R., Y. Pillon, **T. Sakishima**, C. E. C. Gemmill, S. Chamala, W. B. Barbazuk, E. Lucas, S. M. Geib, and E. A. **Stacy**. (2019) Targeted amplicon sequencing of 40 nuclear genes supports a single introduction and rapid radiation of Hawaiian *Metrosideros* (Myrtaceae). *Plant Systematics and Evolution* <https://doi.org/10.1007/s00606-019-01615-0>.

Stacy, E. A. and **T. Sakishima**. (2019) Phylogeography of a highly dispersible landscape-dominant woody species complex. *Journal of Biogeography* 46: 2215-2231  
<https://doi.org/10.1111/jbi.13650>.

Johnson, M., Y. Pillon, **T. Sakishima**, D. Price, and E. Stacy. (2019) Multiple colonizations, hybridization and uneven diversification in *Cyrtandra* (Gesneriaceae) lineages on Hawaii Island. *Journal of Biogeography* 46(6): 1178-1196. (DOI) -010.1111/jbi.13567.

Stacy, E. A., J. B. Johansen, **T. Sakishima**, and D. K. Price. (2016) Genetic analysis of an ephemeral intraspecific hybrid zone in the hypervariable tree, *Metrosideros polymorpha*, on Hawaii Island. *Heredity* 117: 173-183.

Pillon, Y., E. Lucas, J. B. Johansen, **T. Sakishima**, B. Hall, S. M. Geib, and E. A. Stacy. (2015) An expanded *Metrosideros* (Myrtaceae) to include *Carpolepis* and *Tepualia* based on nuclear genes. *Systematic Botany* 40(3) 782-790.

Pillon, Y., J. Johansen, **T. Sakishima**, S. Chamala, W. B. Barbazuk, and E. Stacy. (2014) Primers for low-copy nuclear genes in *Metrosideros* and cross-amplification in Myrtaceae. *Applications in Plant Sciences* 2(10) 1400049.

Stacy, E. A., J. B. Johansen, **T. Sakishima**, D. K. Price, Y. Pillon. (2014) Incipient radiation within the dominant Hawaiian tree *Metrosideros polymorpha*. *Heredity* 113: 334-342.

Pillon, Y., J. B. Johansen, **T. Sakishima**, E. H. Roalson, D. K. Price, and E. A. Stacy. (2013) Gene discordance in phylogenomics of recent plant radiations, an example from Hawaiian *Cyrtandra* (Gesneriaceae). *Molecular Phylogenetics and Evolution* 69: 293-298.

Pillon, Y. J. Johansen, **T. Sakishima**, S. Chamala, W. Brad Barbazuk, and E. A. Stacy. (2013) Primers for low-copy nuclear genes in the Hawaiian endemic *Clermontia* (Campanulaceae) and cross-amplification in Lobelioideae. *Applications in Plant Sciences* 1: 1200450.

Pillon, Y., J. Johansen, **T. Sakishima**, S. Chamala, W. Brad Barbazuk, E. H. Roalson, D. K. Price and E. A. Stacy. (2013) Potential use of low-copy nuclear genes in DNA barcoding: a comparison with plastid genes in two Hawaiian plant radiations. *BMC Evolutionary Biology* 13: 35.

## PRESENTATIONS

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**Sakishima, T.** (2020) Plant communication: Are plants nice to their neighbors? UNLV Rebel Grad Slam, Las Vegas, Nevada.

**Sakishima, T.,** A. Cuttriss, D. K. Price, and E. A. Stacy. (2015) Local adaptation of the Hawaiian endemic tree (*Metrosideros polymorpha*) along a steep elevation gradient. Association for Tropical Biology and Conservation, Honolulu, Hawai'i.

**Sakishima, T.,** A. Cuttriss, D. K. Price, and E. A. Stacy. (2015) Local adaptation of the Hawaiian endemic tree (*Metrosideros polymorpha*) along a steep elevation gradient. Society for the Study of Evolution Conference, Guarujá, Brazil.

**Sakishima, T.,** A. Cuttriss, D. K. Price, and E. A. Stacy. (2015) Local adaptation of the Hawaiian endemic tree (*Metrosideros polymorpha*) along a steep elevation gradient. 7<sup>th</sup> Annual Tropical Conservation of Biology and Environmental Science Research Symposium, Hilo, Hawai'i.

**Sakishima, T.,** A. Cuttriss, D. K. Price, and E. A. Stacy. (2015) Local adaptation of the Hawaiian endemic tree (*Metrosideros polymorpha*) along a steep elevation gradient. The 62<sup>nd</sup> Annual Meeting of the Ecological Society of Japan, Kagoshima, Japan.

**Sakishima, T.,** A. Cuttriss, D. K. Price, and E. A. Stacy. (2014) Local adaptation of *Metrosideros polymorpha* along a steep elevation gradient. Island Biology Meeting, Honolulu, Hawai'i. (poster)

Stacy, E., J. Johansen, A. Rhoades, K. Morrison, **T. Sakishima**, Y. Pillon, J. Ekar and D. Price. (2014) Environmental gradients as drivers of speciation in island trees. Island Biology Meeting, Honolulu, Hawai'i.

Pillon, Y., S. Chamala, W. B. Barbazuk, J. Johansen, **T. Sakishima** and E. Stacy. (2014) Phylogenomics of *Metrosideros* in Hawai'i and the Pacific. Association for Tropical Biology and Conservation, Cairnes, Australia.

Pillon, Y., S. Chamala, W. B. Barbazuk, J. Johansen, **T. Sakishima** and E. Stacy. (2014) Phylogenomics of *Metrosideros* in Hawai'i and the Pacific. Island Biology Meeting, Honolulu, Hawai'i.

**Sakishima, T.**, A. Cuttriss, D. K. Price, and E. A. Stacy. (2014) Local adaptation of *Metrosideros polymorpha* along a steep elevation gradient. Society for the Study of Evolution Conference, Raleigh, North Carolina.

**Sakishima, T.**, A. Cuttriss, D. K. Price, and E. A. Stacy. (2014) Local adaptation of *Metrosideros polymorpha* along a steep elevation gradient. 6<sup>th</sup> Annual Tropical Conservation of Biology and Environmental Science Research Symposium, Hilo, Hawai'i.

Stacy, E. A., J. B. Johansen, **T. Sakishima**, A. M. Rhoades, Y. Pillon, and D. K. Price. (2013) Environmental gradients as drivers of speciation in tropical trees. Association for Tropical Biology and Conservation, San Jose, Costa Rica.

Stacy, E. A., J. Johansen, **T. Sakishima**, K. Morrison, A. Rhoades, Y. Pillon, and D. Powless. (2013) Speciation in trees according to Hawaiian *Metrosideros*. Department of Botany, University of Hawai'i at Manoa.

**Sakishima, T.**, A. Cuttriss, D. K. Price, and E. A. Stacy. (2013) Local adaptation of *Metrosideros polymorpha* along a steep elevation gradient. 5<sup>th</sup> Annual Tropical Conservation of Biology and Environmental Science Research Symposium, Hilo, Hawai'i.

Stacy, E., J. Johansen, K. Morrison, **T. Sakishima**, and Y. Pillon. (2012) The many forms of 'ōhi'a lehua: do they matter? Symposium: *Hawaii's Vital 'Ohi'a (Metrosideros polymorpha) Forests: How They are Doing, and Why We Should Care*. Hawai'i Conservation Conference, Honolulu, Hawai'i.

Pillon, Y., J. Johansen, **T. Sakishima**, and E. Stacy. (2012) Evolution of the Hawaiian endemic genus *Clermontia* inferred from nuclear and plastid SNPs. Joint Meeting of the Society for the Study of Evolution and American Naturalists Society, Ottawa, Canada. [poster]

Stacy, E. J. Johansen, K. Morrison, **T. Sakishima**, and Y. Pillon. (2012) Divergence and the evolution of reproductive isolation within the Hawaiian endemic tree, *Metrosideros polymorpha*. Joint Meeting of the Society for the Study of Evolution and American Naturalists Society, Ottawa, Canada. (poster)

Stacy, E. J. Johansen, K. Morrison, **T. Sakishima**, and Y. Pillon. (2012) Divergence and the evolution of reproductive isolation within the Hawaiian endemic tree, *Metrosideros polymorpha*. Association for Tropical Biology and Conservation, Bonito, Brazil. (poster)

**Sakishima, T.**, F. Stone, D. K. Price, K. Magnacca, A. Raver, D. Na, and J. Eldon. (2012) Population phylogeography of cave-adapted crickets on Hawai'i Island (*Caconemobius*: Gryllidae, Nemobiinae). 4<sup>th</sup> Annual Tropical Conservation Biology and Environmental Science Research Symposium.

Pillon, Y., J. B. Johansen, **T. Sakishima**, S. Chamala, B. Barbazuk, and E. A. Stacy. (2011) Inferring the evolution of *Clermontia* (Campanulaceae) and *Cyrtandra* (Gesneriaceae) in Hawai'i with nuclear gene sequences. Joint Meeting of the Society for the Study of Evolution and American Naturalists Society, Norman, Oklahoma.

Stacy, E. A., J. Johansen, **T. Sakishima**, and Y. Pillon. (2011) *Metrosideros polymorpha* varieties across space and time on Hawai'i Island. Joint Meeting of the Society for the Study of Evolution and American Naturalists Society, Norman, Oklahoma.

Pillon, Y., J. B. Johansen, **T. Sakishima**, S. Chamala, B. Barbazuk, and E. A. Stacy. (2011) Evolution of the genus *Clermontia* (Campanulaceae) in Hawai'i inferred by next-generation sequencing. Evolution of Life on Islands Meeting, Smithsonian Institution, Honolulu, Hawai'i.

Stacy, E. A., N. DeBoer, J. B. Johansen, **T. Sakishima**, and Y. Pillon. (2010) Analysis of population structure reveals dispersal limitation and significant differentiation of extreme-habitat varieties of the dominant *M. polymorpha* on east Hawai'i Island. Annual Meeting of the Botanical Society of America, Providence, Rhode Island.

Stacy, E., W. Barbazuk, J. Johansen, Y. Pillon, **T. Sakishima**, and S. Chamala. (2010) A next-generation sequencing approach to DNA barcoding in plants. Annual Meeting of the American Genetics Association, Hilo, Hawai'i.

Stacy, E., N. DeBoer, J. Johansen, Y. Pillon, and **T. Sakishima**. (2010) *Metrosideros polymorpha*: A partial solution from evolutionary studies on Hawai'i Island. Symposium: *In honor of Dieter Mueller-Dombois*. Vitousek Ecosystems Meeting, Hilo, Hawai'i.

Stacy, E. A., Y. Pillon, **T. Sakishima**, and J. B. Johansen. (2010) Microsatellite analysis of an intraspecific hybrid zone in *Metrosideros polymorpha*. Joint Meeting of the Society for the Study of Evolution and American Naturalists Society, Portland, Oregon.



## **GRANTS AND FELLOWSHIPS**

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UNLV President's UNLV Foundation Graduate Research Fellowship (2021)

UNLV Rebel Grad Slam Scholarship (2020)

UNLV Doctoral Summer Fellowship (2020 & 2021)

UNLV Graduate College Recruitment Scholarship (2016)

Island Biology Conference in Hawai'i Financial Support (2014)

## **VOLUNTEER**

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Judge the High School projects at the Beal Bank USA Southern Nevada Regional Science & Engineering Fair (2021)

- Judged 3 projects in the plant science field

Family STEM Nights to engage middle school students in STEM activities at Kamehameha School in Keaau, Hawai'i (2020)

- Assisted in leading one of activities

## Bibliography

Tomoko earned her first Bachelor's degree in Policy Studies with a focus in Human Ecology in 2005 from Kwansei Gakuin University, Japan. She moved to Hawaii in 2006 and earned her second Bachelor's degree in Biology in 2009 from University of Hawaii at Hilo. While she was working as a lab technician, she earned Master's degree in Tropical Conservation Biology and Environmental Science studying adaptation in Hawaiian endemic trees along an elevational gradient in 2015 from University of Hawaii at Hilo.

She mentored >15 undergraduate and high school students and had four publications during PhD.

Stacy, E. A., **T. Sakishima**, H. Tharp, and N. Snow. (2020) Strong isolation within *Metrosideros* ('Ohi'a) on O'ahu correlates with extreme environments. Symposium Issue; Journal of Heredity 111: 103-118.

Dupuis, J. R., Y. Pillon, **T. Sakishima**, C. E. C. Gemmill, S. Chamala, W. B. Barbazuk, E. Lucas, S. M. Geib, and E. A. **Stacy**. (2019) Targeted amplicon sequencing of 40 nuclear genes supports a single introduction and rapid radiation of Hawaiian *Metrosideros* (Myrtaceae). *Plant Systematics and Evolution* <https://doi.org/10.1007/s00606-019-01615-0>.

Stacy, E. A. and **T. Sakishima**. (2019) Phylogeography of a highly dispersible landscape-dominant woody species complex. *Journal of Biogeography* 46: 2215-2231 <https://doi.org/10.1111/jbi.13650>.

Johnson, M., Y. Pillon, **T. Sakishima**, D. Price, and E. Stacy. (2019) Multiple colonizations, hybridization and uneven diversification in *Cyrtandra* (Gesneriaceae) lineages on Hawaii Island. *Journal of Biogeography* 46(6): 1178-1196. (DOI) -010.1111/jbi.13567.

Tomoko's research has been supported by a number of fellowships including President's UNLV Foundation Graduate Research Fellowship (2021), Rebel Grad Slam Scholarship (2020), Doctoral Summer Fellowship (2020 & 2021), and Graduate College Recruitment Scholarship (2016).