EXPLORING FACTORS THAT INFLUENCE THE ROOTING SUCCESS OF *ACACIA KOA* STEM CUTTINGS

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INTRODUCTION

A. Overview

Acacia koa is a native Hawaiian tree that has proven to be an important for regional ecology, local economy, and island culture. Koa has been propagated for restoration purposes, however there is a growing demand to meet other forestry objectives on public and private lands. Over the past few decades, there has been a growing interest in koa silviculture in response to the increased market price of high-value timber. Presently, koa timber consists primarily of old-growth trees harvested from private lands, including dying and dead trees salvaged for useable wood.

Thus far, studies have shown that a high amount wood quality in koa trees within and between populations and not all koa will inevitably produce the desired wood grain and coloration associated with desired characteristics (Atchinson, 1948). Therefore, there is a need to identify drivers of wood quality and develop a robust tree breeding improvement program. This begins with the selection of elite stock, followed by an improvement the propagation techniques and silvicultural practices used to create superior timber trees.

The Tropical Hardwood Tree Improvement and Regeneration Center has been working on related projects and the development of tree improvement programs for koa has been made a priority (www.TropHTIRC.com). This study aims to create a foundation for tree improvement by investigating propagation techniques for this species. An important component of these programs is to use vegetative propagation methods to produce genetically identical stock plants and seed trees. Using single genotypes can improve the understanding of the heritability of select traits, reduce impacts caused by other environmental factors, and create superior trees for perpetuating desired characteristics (Willan, 1988). This allows for the development of programs

aimed at cultivating quality tree stock (Keiding and Graudal, 1996). Vegetative propagation is an integral part of these programs because it allows breeders to manipulate and exploit the natural variation that already exists in species without introducing more developmental, environmental, and genetic influences (Willan, 1988). These programs have been prevalent in traditional forestry practices and provide cost effective ways to grow and manage trees (Zobel and Talbert, 1984).

The following studies aim to elucidate factors that influence rooting success in this difficult to root species by exploring a few of the many factors that influence adventitious rooting in stem cuttings.

B. Species Profile

Koa is a leguminous tree species that, along with hundreds of other native species, are endemic to the Hawai'ian islands. This pioneer species dominates canopies in upland mesic forests and naturally occurs across a variety of precipitation regimes, elevations, and soil types (Baker et al. 2009). Koa forests are integral to the landscape and the various stages of succession provide conditions to support native plant and animal communities (Pejchar and Press, 2006).

In addition to the ecological benefits that make this species valuable, koa also has significant economic value. The wood is extremely marketable due to its unique coloration and curly figure that has multi-dimensional aspects (Jenkins, 1983). Koa is the greatest contributor to the state's $US 30 million forest industry and is one of the most valuable timber species in the world (Yanagida et al. 2004, Friday et al. 2006).However, demand for koa wood products is much greater than the amount of raw materials there is to supply the market (Jenkins, 1983; Loudat and Kanter, 1997). Koa has previously been viewed as an extractable resource starting when major land use changes in the mid-1800s greatly altered koa forest structure and

distribution throughout its range (Cuddihy and Stone, 1990). Land was converted from native forests to farmland for high-yield crops and pastureland for cattle. Naturally regenerated koa were suppressed due to grazing, causing a precipitous decline in the area of koa forest. Consequently, only a fraction of the historical extent remains today (Barrera and Kelly, 1974; Wilkinson and Elevitch, 2003).

Today koa forests are being restored on public and private lands through large outplantings of seedlings, as well as through the promotion of natural regeneration from existing seed banks and resprouting. In the past, koa seedlings used in these efforts have come from seeds collected from local trees. Koa naturally has a high degree of genetic diversity which could be exploited to select families with certain characteristics from populations across its existing range, however capturing these genetics has proven difficult. Koa is an open-pollinated species with 4 sets of chromosomes creating high genetic and phenotypic diversity within and between populations (Atchinson, 1948). The koa flower is highly dichotomous and the pollen is released from the anther days before the stigma and style have finished uncoiling. Therefore, it is speculated, that even though the tree could self-pollinate, it does not due to the timing of the flower's cycle creating seeds that only share up to 50% of their genetic make-up (Brewbaker, 1977).

The integrity and conservation of local genetics has been pursued primarily through collection of seeds from existing trees in the same area. In addition to the great deal of genetic diversity that exists within the species, seed collection from natural stands also poses a problem because (i) seed production varies with location and annual weather patterns; (ii) many koa populations are in remote locations; (iii) and pest and disease pressures generally lead to high seedling mortality at a low elevation (Daehler and Dudley, 2002; James et al 2007). These

factors have slowed progress in tree breeding improvement programs. An alternative is to select material for traits such as wood quality, local adaptations, disease resistance, growth rate, bole straightness, crown architecture, and seed production can be accomplished through clonal propagation methods.

C. Tree Improvement

Tree improvement programs aim at perpetuating the genetics of superior trees in order to produce marketable timber in the shortest period of time at a reasonable cost (Zobel and Talbert, 1984). These tree improvement programs aim to optimize the cultivation of quality trees in which successive generations are suited to the needs of the management objective by selecting for desirable attributes (Keiding and Graudal, 1996). Not only does this have financial incentive, but also restoration incentive as it can improve the ecosystem conditions to support native plant and animal communities (Pejchar and Press, 2006).

Tree improvement programs begin with the selection of elite individual trees. After these individuals have been identified, seeds or vegetative material is collected in order to capture the desired traits. Selection typically occurs based on how the tree appears; however, the phenotypic expression is the result of genetic and environmental interactions. Therefore, isolating genetic influences is required to better understand the heritability of select traits and reduce impacts caused by other factors (Willan, 1988).

The most effective way to do this is by multiplying a single genotype through clonal propagation. This allows researchers to examine how the environment influences plants that all have the same genetic makeup. Tree improvement programs based around clonal propagation can increase productivity, product quality, and lower production costs and has become an important mechanism for increasing success in forest industry (de Assis, 2001). The ability to

tailor growth characteristics to management objectives is financially beneficial as well as aims to ensure the continuation of a species throughout its native range. Koa has displayed a great deal of morphological variation, especially in relation to tree form, leaf, seed, and flower characteristics. This necessitates the ability propagate locally-adapted clones for successful outplanting across its historic range as well as for conserving genetic diversity within the species (Sun et al, 1997).

D. Vegetative Propagation

Vegetative propagation, also known as clonal propagation, is a method of producing genetically identical copies of the parent plant. This differs from propagation by seed that produces offspring that are a recombinant of the mother plant and pollen donor. Although traditional methods by seed have resulted in high yield, there are disadvantages that include:

- Seasonality of seed collection and uncertainty as how many can be collected due to variability in flowering and seed production.
- Variability in favorable traits from trees that are collected from the same mother tree (half-sibs)
- Limited knowledge about the heritability of favorable traits
- Time required for outplanted trees to reach maturity and start producing seeds
- Offspring may not possess same characteristics as parent trees (Monteiuss and Maitre, 2007)

Vegetative propagation captures favorable genetics to improve existing tree populations while achieving greater numbers of uniform, high quality material and overcoming barriers presented through seed propagation (Leakey, 2004). Success of this method is dependent upon the ability of the stock plant to create shoots that will produce adventitious roots, as well as

displaying minimal within-clone variation by developing true-to-type (Rauter, 1983; Libby and Rauter, 1984). Ideally, this propagation strategy leads to the retention of desirable genetics that were selected for in the field. The total natural genetic variance is captured, potentially maximizing the benefits of wood quality and productivity through the production of a uniform raw material (de Assis, 2001).

There are different methods of vegetative propagation, each requiring a different level of skill and technology (Table 1).When optimized, vegetative propagation can rapidly achieve large numbers of uniform, high quality clonal material while overcoming barriers presented through seed propagation (Monteuuis and Maitre, 2007). The use of vegetative propagation as the main driver of tree improvement programs have been successfully applied to other valuable tropical hardwoods, most notably *Eucalyptus* spp. and *Tectona grandis* (de Assis, 2001; Goh and Monteiuss, 2005). These efforts resulted in well-established planting programs structured around selecting, multiplying, and screening individual genotypes.

Table 1. A comparison table of clonal propagation methods with each technique ranked in relation to one another. (1=easiest/least and 4=most difficult/ greatest).

	Level of Skill	***Cost of Supplies + Equipment***	***Types of Starting Material***
Tissue Culture	4	4	Small pieces of plant tissue
Grafting	3	3	Seedlings, Stems (Juvenille or Mature)
Stem Cuttings	2	2	Seedlings, Resprouts, Stems (Juvenille)
Air Layering	1	1	2-8cm Intact Stem

It is imperative to be able to produce mass quantities of single genotypes so that research activities and screening trials are robust and indicative of how (i) specific clones respond to the environment and (ii) the heritability of desirable genes (Leakey, 2004). Clones expedite the process of selection because there is a better understanding of how individual genotypes will respond under test or field conditions, as well as uniformity of desirable attributes (Zobel and Talbert 1984). By utilizing techniques such as rooted cuttings, a single genotype can be

multiplied, screened, and improved upon through selection (Zobel and Talbert, 1984). Ideally, these superior genotypes will be out planted in a seed orchard allowing select trees to cross with one another.

Although this process seems straightforward, poor rooting success can make it difficult to test large numbers of single genotypes and can greatly limit clonal yield. A koa tree improvement program, initiated in 1973 by the US Forest Service, attempted to employ vegetative propagation methods. Rooting success differed considerably across vegetative propagation techniques, but overall success was low with the most successful method only yielding 3000 mg/L rooting success (Skolmen, 1977).

Confounding environmental and genetic factors are responsible for low rooting success and rooted cutting survivability, but the extent to which each plays a role is largely unknown (Skolmen, 1977; Sun and Brewbaker, 1996). Due to the low rooting success of koa, obtaining select material from superior trees in the field would be difficult. Therefore, the material that is gathered from the base population must be propagated using proven methodologies in order to ensure the longevity of favorable genetics.

E. Factors that Influence Rooting Success

The factors that influence rooting success are numerous and interactive making it difficult to determine the ideal materials and conditions for vegetative propagation, as well as the underlying physiological mechanism driving root production (Leakey, 2004).

Table 2. Factors that influence rooting success.

Pre-Severance	Cutting Attribtues	Propagation Environment
Source of Starting Material	Leaf Area	Light
Condition of Starting Material	Auxin Application	Humidity
Stock Plant Environment	Degree of Lignification	Temperature
Stock Plant Nutrient Management	Stem Length	Cleanliness
Stem Position		Rooting Media
Juvenility		
Genetics		

i. Pre-severance

The pre-severance condition of starting material is the first critical factor to consider in determining the rooting ability of stem cuttings (Table 2). The material should appear healthy (i.e. pest-free, no signs of nutrient deficiencies or toxicities, normal growth and development, etc.) whether it is collected from managed stock plants in a nursery of from wild populations. Stem position is important because there are many within shoot factors that affect adventitious root initiation resulting in physiological differences between the proximal to the distal end a stem. These include: leaf size, leaf water potential, leaf carbon balance, leaf senescence, internode length, internode diameter, stem carbohydrate content, etc. (Leakey and Mohammed, 1985)

Juvenility has repeatedly proven to be a critical factor in rootability of many woody plant cuttings and is easily identified by a basic understanding of leaf morphology (Kormanik and Brown, 1974; Skolmen 1977). True leaves of koa are bipinnately compound, indicating a juvenile plant. As the plant matures, the leaves transition to phyllodes, and rooting ability of stems is low (Shi, 2006)

The degree to which genetics plays a factor is largely unknown. A study conducted by Shi (2006) suggested that within family variation of rooting success was large, prompting the need to explore ways to increase rootability across genotypes. Therefore, it is important to better

understand that factors that influence rooting success in order to increase rootability across genotypes.

ii. Cutting Attributes:

Other factors that can influence the rooting ability of cuttings are the morphological and physiological attributes of cuttings (Table 2). It is imperative to minimize water stress experienced by the leaves and to maximize photosynthetic activity, both of which have proven to aid in the production of new roots (Leakey, 2012). Especially because leafy stem cutting from nonlignified shoots rely on assimilates produced while in the propagation bed (Leakey, 2004). Therefore, morphological characteristics of the cutting play a role in root production including leaf size, internode length, and within shoot position. This is due to the age gradient from growing tip to basal portion of the stem which translates to differences in carbohydrate, nutrient, and water content within the shoots (Leakey, 2012).

The degree of lignification also influences rooting success. More mature stems have greater amounts of lignin making them "woodier", which makes it harder for roots initials to penetrate tissue (de Assis et al, 2004). Difficult to root species, such as koa, typically have heavier lignification of the phloem fibers, more sclerid development, and a less active cambium (Hartmann and Kester, 2010).

iii. Propagation Environment

Rooted cuttings are very sensitive to post-severance factors in the propagation environment and are fragile until they have established a root system. Prior research indicates that complex interactions between the nutritional status of the starting material and the quality and quantity of light supplied to the cutting play a major role in rooting success. This is because these factors influence the photosynthetic capacity and carbohydrate status of the cutting (Leakey

and Storeton-West, 1992). The environment must promote photosynthesis, transpiration, and meristematic activity while minimizing stress experienced by the severed plant tissues in order to enhance rooting success (Leakey, 2004).

CHAPTER 1: ROOTING SUCCESS OF *ACACIA KOA* STEM CUTTINGS COMPARING AUXIN CONCENTRATION, STEM POSITION AND POST SEVERANCE LIGHT LEVEL

Background

Acacia koa is a native Hawaiian tree that is an important component of the ecology of forested landscapes and the state's economy (Baker et al 2004). Koa populations have quickly declined due to various anthropogenic pressures such as land conversion and the introduction of invasive species that outcompete native plant and animal communities. In addition, this tree is a major contributor to the state's $30million year timber and forest products industry (Yanagida et al, 2004). Historically, koa has been seen as an extractable resource, but is now shifting towards more sustainable forestry practices to meet the needs of multiple objectives (Baker et al, 2009). Tree improvement programs are a way to capture desirable characteristics such as growth form, wood quality, disease resistance and local adaptations. In addition, selectively breeding for superior trees will help koa overcome anthropogenic pressures. These programs can achieve quicker gains by using vegetative propagation techniques to generate genetically identical material (Zobel, 1984). The most effective way to achieve superior genetics is by selecting elite trees and then using vegetative propagation to obtain identical copies (Zobel, 1984). However, some species are very difficult to root and research must be conducted to understand how to overcome these barriers. There are many factors that play into the production of adventitious roots and can include source and health of stock plants, cutting attributes, post-severance treatments, and the propagation environment. Koa is one of these notoriously difficult to root species and overcoming this barrier is vital to meet all management objectives.

There are many aspects of cutting material and propagation environment that can be controlled in order to achieve higher rooting success (Table 2). The application of auxin is the

most common post-severance treatment in clonal propagation and generally improves root quality in terms of number and length of the roots produced (Cerveney and Gibson, 2005; Dirr 1978). Auxin is a growth regulating phtyohormone that is used to promote root initiation by stimulating cell differentiation and the mobilization of vital nutrients and sugars to the base of the cutting. Using a synthetic auxin induces adventitious root formation more rapidly and with higher success (Hartmann et al, 2010; Das et al. 1997). The dilution of the rooting hormone depends on the species and requires experimentation to determine the optimal concentration. Too little exposure to auxin will result in low rooting success while overexpose can result in burning or dehydrating sensitive plant tissues (Cerveney and Gibson, 2005). Even if there is no mechanical damage sustained by the cutting tip from too high an auxin concentration, there is a physiological change in which auxin transitions from promoting root development to inhibiting root development after 96 hrs (De Klerk et al 1990). Therefore, too high of a concentration could lead to an inhibitory response from the cutting. Previous work has indicated that koa is sensitive to different concentrations of liquid rooting hormone solutions. A study conducted by Skolmen in 1977showed that true leaf cuttings had the greatest likelihood of producing roots when exposed to a 3000 mg/L solution of auxin and stuck in perlite. The concentration of auxin used in this study ranged from 10 mg/L to 3000 mg/L, with the upper limit having the greatest percentage of true leaf cuttings rooted (Skolmen, 1977). For the purpose of the current study, a rooting hormone concentration that exceeded this upper limit was used to determine if efficacy improved past the 3000 mg/L limit that was previously tested.

Controlling the propagation environment in order to reduce the shock experienced by the cuttings is also critical to the success of root formation (Leakey, 2004).A general guideline for stem cuttings of tropical trees suggests that root development is most likely to occur in a

propagation environment that provides moderate temperatures (23-27°C) and high humidity (>85%). The easiest way to adjust temperature and humidity is by controlling the amount of light and moisture that reaches the cuttings that can be achieved by shading the propagation area (Leakey et al 1990). An ideal propagation environment has enough light to stimulate physiological (i.e. photosynthesis and transpiration) and meristematic activity while minimizing water stress experienced by the cutting (Leakey, 2004). Too little light can stifle growth by slowing metabolic activity and cause conditions for pathogens to infect an already stressed stem cutting. Shade helps moderate temperature and keep humidity high providing a low vapor pressure deficit to the leaf surface subsequently lowering the overall amount of stress experienced by the cutting. In addition, shade can be used to regulate the physiological processes in the cutting. Reid et al. 1991 suggests shading can affect the hormone metabolism and the sensitivity of cells to auxin. In *Phaseolus aureus*, it was observed that more light promotes root initiation in the presence of exogenous auxin, but will inhibit root development if rooting hormone is *not* applied post-severance. Furthermore, higher light levels promoted the uptake of exogenous auxin due to higher transpiration rates (Jarvis and Ali, 19894; Jarvis and Shaheed, 1987).

The within shoot position is an important factor to explore because it dictates the physiology and morphology of the cutting, thus the ability for it to root. An age gradient exists between the tip and base of the stem and influences leaf size, degree of lignification, internode length, nutrient and stem carbohydrate content, and many other morphological and physiological characteristics that could influence rooting success (Leakey, 2004). Cuttings located closer to the tip are closer to the main source of auxin biosynthesis in the plant and theoretically should possess a higher propensity to root (Hartmann and Kester, 2010). Previous work showedthat

basal stem cuttings of *Khayaivonesis* had a greater rooting percentage across treatments and clones than those collected from apical nodes (Tchoundjeuet al, 1995). Conversely, Poupard (1994) found that the greatest percentage came from the second position in stems of *Acacia mangium* with the lowest rooting percentage occurring in cutting taken from the base of the stem. This contradiction captures the species-specific intricacies of rooting success and that it is determined by a combination of factors.

This experiment focuses on a collection of starting material from naturally regenerated root suckers in order to capture the genetics of trees from the field. Taking cuttings from juvenile shoots of recently harvested koa trees has been shown to be possible, but low success overall (Skolmen, 1977).Two critical factors, rooting hormone treatment and the propagation environment, were assessed simultaneously.

Hypotheses

[1] A rooting hormone solution that has a concentration greater than 3,000 mg/L of auxin (recommended) will promote higher rooting success.

[2] Stem cuttings located closer to the apical meristem will have greater rooting success than those located towards the basal end.

[3] Koa stem cuttings propagated under the 50% shade will have greater rooting success than those propagated under 90%.

MATERIALS AND METHODS

Propagation Area Set-up and Cutting Collection

The plant material for stem cuttings came from two naturally regenerated stands of koa root suckers. These field sites were Pu'u Huluhulu (19°22'26.00"N, 155°12'30.00"W, 2040m elevation) and the koa buffer zone managed by the US Fish and Wildlife Service located above

Hakalau Forest (19°47'37.29" N, 155.19'34.73" W, 1950m elevation) located on Hawai'i Island. Healthy root suckers were severed 6-8 nodes (i.e. well below where the cuttings were taken) below the apical meristem and wrapped in a saturated paper towel with the base of the stem sealed in a Ziploc bag Cuttings were collected between May and October of 2014, however is an evergreen, and cuttings could have been collected all year long.

A thin cloth barrier was placed between the bags and the ice to ensure that no direct contact was made in order to prevent damage to sensitive tissues. After all the cutting material was collected, it was transported back to the propagation area and transferred from the cooler to water-filled bucket. Cuttings were trimmed down to three nodes before placing the cut end into the rooting media. The top node had the leaves still intact and the subsequent nodes were defoliated. The succulent, apical meristem was removed and the first set of leaves was reduced in size by 75 %. The second and third nodes were soaked for 5 seconds in the rooting hormone Dip n Grow™ solution. The concentrate of the rooting hormone contains 1 % indole-butyric acid IBA and 0.5% 1-napthaleneacetic acid NAA) and was diluted with water to create different concentration of the rooting hormone in solution (Figure 1, Table 3). Stem cuttings were immediately inserted into a rooting media that consisted of 2 parts perlite to 1 vermiculite in Ray Leach Cone-Tainer™ SC7 planting tubes supported in 98 cell trays. Cuttings were randomly assigned to a set of treatments and placed in the tray accordingly leaving space between every other cell in the tray so that leaves did not touch. There were four rounds of experiments in total. Round 1 (n=485) and round 2 (n=428) examined rooting hormone concentration and the two treatment levels consisted of solution concentrations of 80% water to 20% Dip n Grow (2,000 mg/ L of IBA and 1000 mg/L of NAA) by volume and a higher concentration of 25% water to 75% Dip n Grow (6700 mg/L of IBA and 3300 mg/L of NAA). The rooting hormone

concentration treatments tested during round 2 were 80% water to 20% Dip n Grow (2,000 mg/L of IBA and 1000 mg/L of NAA) and a 50% water to 50% of Dip n Grow 5000 mg/L of IBA and 2500 mg/L of NAA). After concluding that there was a significant differences between the 3,000 mg/L and 10,000 mg/L treatments in round 1, the solution concentration was changed to a concentration of 7500 mg/L for round 2.

Shade boxes were constructed to house two trays of cuttings. Shade cloth that blocked 50% light transmission and 90% light transmission were used to construct the different shade boxes, and there was one of each shade level per one of the 4 experimental blocks (n=8). Each shade house had raised 1.0mm A.M. Leonard mist head the center to supply a mist that would maintain high humidity and a low vapor pressure deficit around the leaf surface. Temperature and RH were recorded with a HOBO U23 Pro v2 Temperature/Relative Humidity Data Loggers sensor with a built-in data logger that was programmed to collect data every 5 minutes after launch. Environmental conditions were monitored and the misting frequency and duration was adjusted to maintain high humidity (>90°) and moderate temperature (<85°). There were 4 replications of each of the shade treatments that was included as a block effect in the statistical analysis. Cuttings were randomized by rooting hormone concentration within the tray and allowed at least 1 month to root before rooting success was determined, unless the condition of the cutting was deemed unable to survive (all leaves had abscised and stem rot was visible). After the end of the rooting duration, the presence or absence of roots was recorded for each cutting.

Round 3(n=402) and round 4(n=344) tested stem position and of the experiment, removing the rooting hormone concentration comparison from the equation and employing a fixed application rate at 3,000 mg/L. The stem position of the cutting was introduced as an

experimental factor and included two levels: apical cutting taken closer to the shoot tip, and basal, a cutting that was taken closer to the basal end of the starting material. The cutting was preparedin a similar way to the previous round. The succulent tip was removed, the first node included and intact leaf pair, and the subsequent 2 nodes were defoliated and submerged in rooting hormone solution. However, two cuttings were taken from the original stem. The apical cutting consisted of nodes 1-3 of the original stem and the basal cutting consisted of the nodes 4-6.

Table 3. Rooting hormone solutions and corresponding percentage and mg/L in solution of the compounds IBA and NAA.

Rooting Hormone Solution	***% Dip n Grow***	***%Water***	***IBA mg/ L***	***NAA mg/L***	***Combined mg/L***
1:4 (Recommended)	20	80	2000	1000	3000
1:1	50	50	5000	2500	7500
2:1	67	33	6700	3300	10,000

Figure 1. a) Example of root sucker from which cutting material was collected with cut lines b) stem cutting that is ready to be placed in propagation environment.

Figure 2. Shade box containing two trays for cuttings and a raised mist head.

Statistical Analysis

A generalized linear mixed model approach was used to analyze the data. and was the most appropriate method for binary data that had categorical variables. These models provide an approach that is better designed to handle non-normal (i.e. binary) data with random effects. It combines the generalized linear models that are able to handle nonnormal data with those of linear mixed models which allow for the incorporation of random effects and is a way to quantify the amount of variation that occurs among units.

For this experiment, the fixed factors were the rooting hormone concentrations and shade level. The random effects included block and the interaction effect between shade level and block. The simplest equation for the model is y~a+b+(1|c)+1(c:b) where y is the dependent variable, a and b are the independent factors, (1|c) is a random effect and (1|c:b) is a random effect created by the interaction of two variables. A model that included all of the factors and random effects was fit using a maximum likelihood by a Laplace approximation. Models for each combinations of factors and random effects were then created and compared using a likelihood ratio test. This was done by comparing a model in which the formula included the

random and a formula that did not include the factor. For example, to test the significance of the rooting hormone solution concentration, an equation that included all of the variables was compared to an formula that contained all of the variables except the rooting hormone concentrations. This was done in the statistical analysis software R 3.2.5 using the lme4 package to generate the model and the car package to acquire the results of the likelihood ratio test. The model comparison allowed for the systematic elimination of variables that were not significant. The effects of a particular factor was considered statistically significant if the p-value produced by performing the likelihood ratio test was less than α=.05.

Table 4. Levels for factors included in each of the 4 rounds of this experiment.

Levels for Factors Included in Each Round			
	Shade Level	*Rooting Hormone Solutions*	*Stem Position*
Round 1	50, 90	10,000 mg/L, 3,000 mg/L	Apical
Round 2	50, 90	7,500 mg/L and 3,000 mg/L	Apical
Round 3	50, 90	3,000 mg/L	Apical, Basal
Round 4	50, 90	3,000 mg/L	Apical, Basal

RESULTS

Overall, the meeting rooting success was higher for the 3,000 mg/L rooting hormone solution under both shade treatments. At the 50% shade level, the 3,000 mg/L solution had higher rooting success than the 10, 000 mg/L solution with means of 3.4% and 0.8%, respectively. Similarly, at the 90% shade level the 3,000 mg/L solution was also higher than the 10,000 mg/L solution with rooting successes of 6.2% and 1.8%, respectively (Figure 3). Through generalized linear model comparison, this factor was deemed significant by a likelihood ratio test in which the p-value was .0101 suggesting that the 3,000 mg/L rooting hormone solution was more effective than the 10,000 mg/L. This was the only factor that influenced rooting success for round 1 and round 2 therefore shade level, block, and the combined interaction of these factors had no significant effect on rooting success.

For round 2, the mean rooting success for 50% shade level was 3.5% for the 3,000 mg/L solution and 4.8% for the 7,500 mg/L solution at the 50% shade level. At the 90% shade level, they were 5.2% and 2.5% respectively (Figure 4). The mean rooting successes for the 3,000 mg/L treatment vs. the 7,500 mg/l were not statistically different from one another as show in the likelihood ratio test where the p value was calculated to be 0.8222 (Table 5). There was also a high degree of variability in the data for this round, as seen by the large standard error in figure 4.

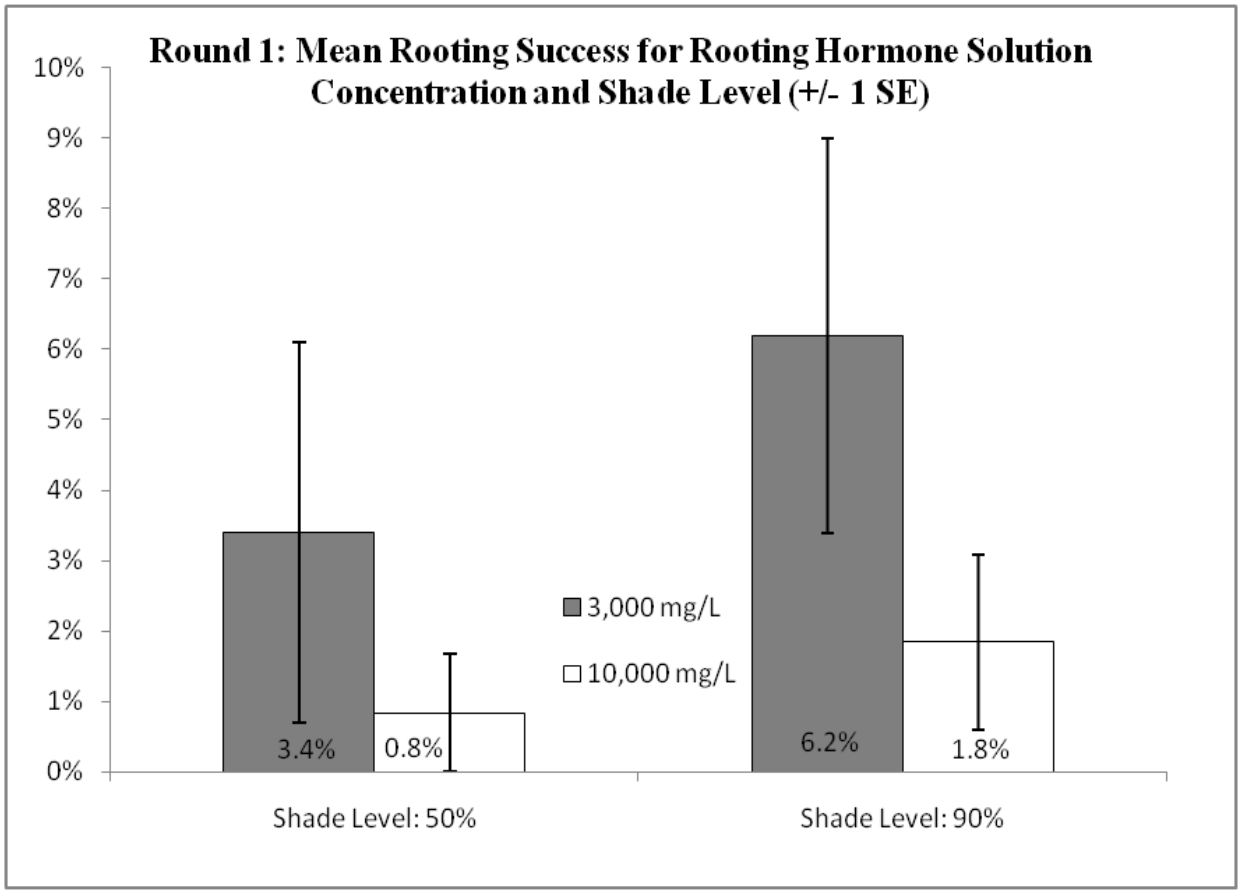

Figure 3. Mean rooting success for 50% and 90% shade level and 3000 mg/L and 10,000 mg/L solutions of rooting hormone treatments labeled with +/- 1 standard error (n=485).

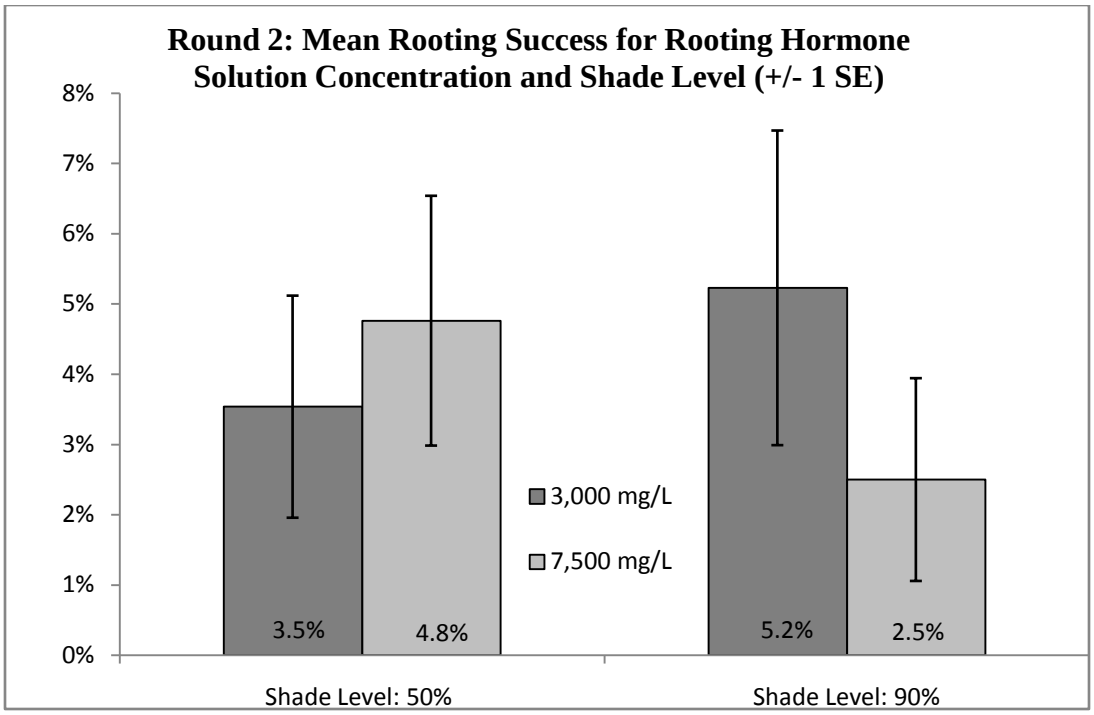

Figure 4. Mean rooting success for the 50% and 90% shade level and 3000 mg/L and 7500 mg/L solutions of rooting hormone treatments labeled with +/- 1 standard error (n=428)

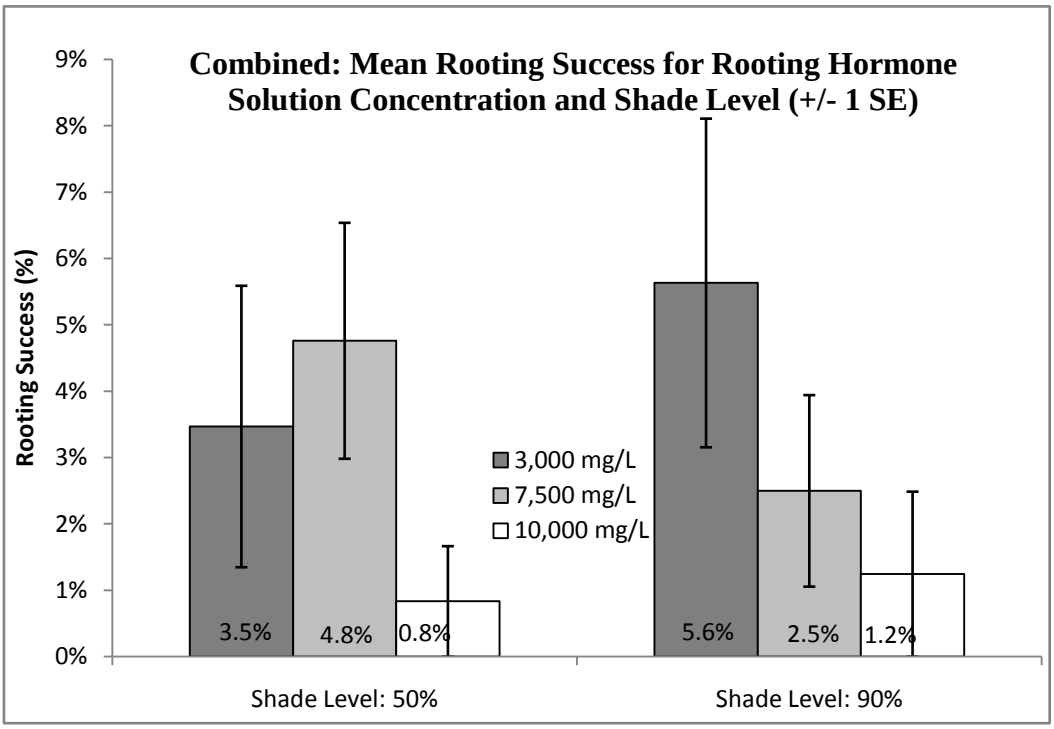

Figure 5. Combined mean rooting success for the 50% and 90% shade levels and the 3000 mg/L (weighted means), 7500 mg/L and 10000 mg/L of rooting hormone concentration treatments (+/- 1 standard error) (n=913).

Table 5. Likelihood ratio test for the shade level and rooting hormone concentration (α=.05).

Likelihood Ratio Test for Fixed and Random Effects				
	Effect Type	*Chi Sq.*	*Df*	*Pr(>ChiSq)*
Rooting Hormone Concentration	Fixed			
Round 1		6.6124	1	0.01013*
Round 2		0.0505	1	0.8222
Combined		6.181	1	.0129*
Shade Level	Fixed	1.353	1	0.2448
Block	Random	1.319	1	0.2509
Block:Shade Level	Random	0	1	0.9998

The results from round 3 and 4 were combined because neither round had any significant experimental factors. A the 50% shade level, the apical cuttings had an average rooting success of 5.0% and the basal cuttings has a success rate of 3.9%. Similarly low results were observed for the 90% shade level as well where 8.9% of apical cuttings rooted and 6.0% of basal cuttings. There was a higher degree of variability in the data as observed by the large standard error bars (Figure 6).

The likelihood ratio test that was used to compare models created from the combined data of round 3 and round 4. Based on the observed p values, the data suggest that there is no significant difference between the different levels of each factor. Most notably, stem position did not seem to have any impact on rooting success.

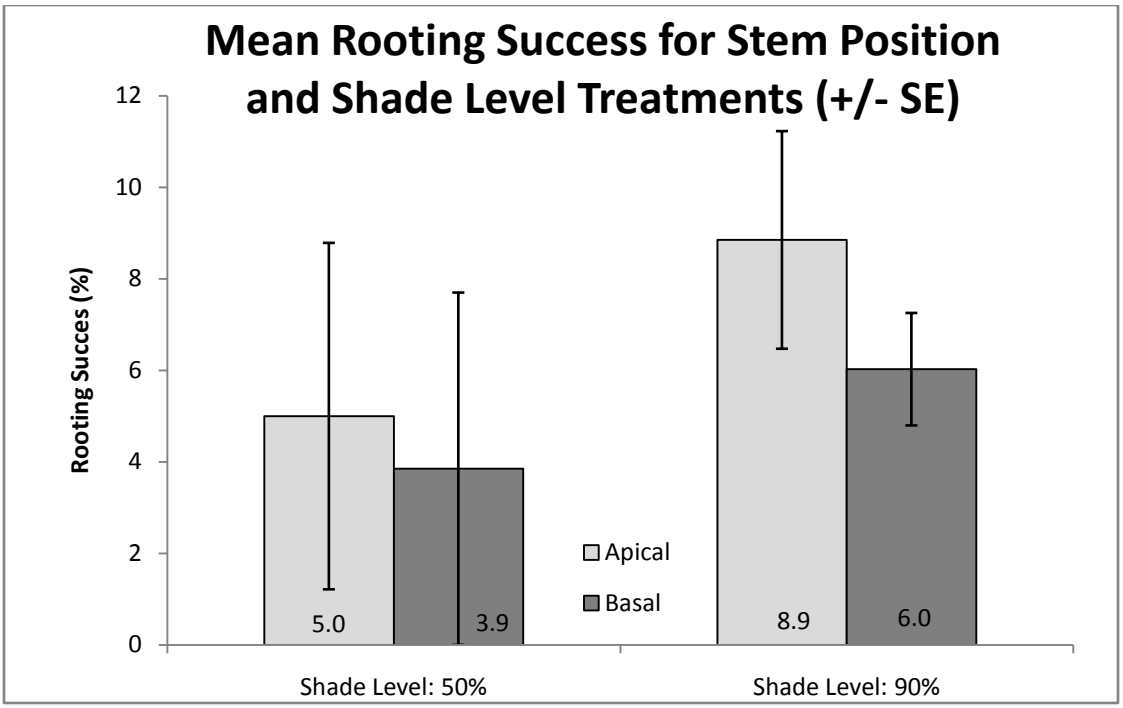

Figure 6. Mean rooting success for shade level and stem position treatments (+/-1 standard error) (n=746).

Table 6. Likelihood ratio test for the shade level and stem position treatment experiment (α=.05)

Likelihood Ratio Test for Fixed and Random Factors				
	Effect Type	***Chi Sq.***	***Df***	***Pr(>ChiSq)***
Stem Position	Fixed	0.7052	1	0.401
Shade Level	Fixed	1.5185	1	0.2085
Block	Random	0	1	1
Round	Random	22.339	1	2.29E-06

DISCUSSION

The many factors that influence rooting success are difficult to study because unless the experiment is being conducted under controlled conditions, there are random effects that could influence the rooting ability of cuttings. These studies aimed to prioritize the factors that help increase rooting success based on recommendations from the literature. The propagation environment, the application of rooting hormone solution at the optimal concentration, and the

position within the stems were suggested to by the most critical. Arguably the most important part of taking cuttings is providing a propagation environment that stimulates and promotes rooting success after it has been severed from the stock plant. During this fragile time, cuttings are prone to environmental stress from which they cannot rebound due to the lack of a healthy and functioning root system. As mentioned, the post-severance environment has to allow the sensitive plant material to balance transpiration and photosynthesis. Fluctuations in temperature, humidity, and light can have drastic effects on rooting success and can be detrimental if the environment is not maintained between a narrow margin of temperature and humidity. Therefore, the confounding factors introduced from environmental fluctuations could play a significant role in how the cutting responded to the stem position and rooting hormone concentration . The greenhouse used at the Komohana Research Station was an open air facility that consisted of shade cloth walls and a rood made of plastic sheeting. The fluctuation of weather conditions throughout the day and night had a major impact on the conditions in the propagation environment and could have destabilized the cuttings condition enough to where they were unable to produce roots. It was common to observe daytime temperatures that often exceeded 37°C. Therefore, the shade levels had to be relatively high in order to reduce the temperature and increase the humidity enough that the cuttings were experience heat stress. This is a possible explanation as to why rooting success was very low for this study (in comparison to Skolmen, 1978). Another factor that could have contributed to the poor rooting success was the irrigation system installed to maintain humidity. This system was programmed to turn on at a fixed interval and did not self-regulate to adjust to changes in temperature or humidity cause by ambient conditions. Additionally, an overhead sprinkler system was used to supply moisture to each of the shade boxes. This system promotes high humidity from the evaporation of water off of the

leaf and soil surfaces when ideally the vapor pressure deficit around the leaf should be maintained by a fine mist humidifying the air. The sprinkler irrigation appeared to have caused periods throughout the day when the cuttings are exposed to cycles of desiccation and oversaturation, ultimately causing more stress to the plant tissue.

Auxin application was another experimental factor chosen because of its documented ability to increase rooting success for a variety of species. The recommended amount of 3,000 mg/L was used in comparison to higher amounts (i.e. 7,500 mg/L amd 10,000 mg/L) to observe if the greater amount of auxin helped to stimulate root production. For the first round of experiments, the high concentration was chosen to try and establish and upper threshold for the solution concentrate working under that assumption that very concentrated amounts would damage sensitive plant tissue causing none of the stem cuttings treated to form roots (Hartmann and Kester, 2010). Due to the significant of results from the first round of rooting experiments suggesting that the higher concentration of 10, 000 mg/L had a lower rooting success rate that the 3,000 mg/L solution prompted the treatment to be changed to more dilute solution of 7,500 mg/L for round 2. The results from this round suggests that there is no significant difference between the 3,000 mg/L and the 7,500 mg/L level. Therefore, for future studies, the 3,000 mg/L concentration was used so that no rooting hormone concentrate was wasted.

Another factor that is linked to the phytohormone auxin is the stem position. Naturally occurring auxin is found at higher concentrations at the tip of the stem than towards the base (Hartmann and Kester, 2010). This is due to physiological and morphological changes that occur along the gradient form the growing tip to the base of the main stem in which tissue becomes more differentiated and ontogenetically older as you move down the gradient (Leakey 2004). In both round 3 and round 4, it was hypothesized that the more apical positions would have a

greater chance of rooting. However, the results indicated that there was no difference between the rooting ability of the two cuttings. This could be that the effects of stem position were masked by the variability that was introduced through other factors for which data was not collected.

The source of cutting material is something to consider, although its effects were not directly tested in this study. Stem cuttings for this experiment were collected from naturally regenerated populations of koa in which important factors such as age, nutritional status, and genetics were completely unknown. Although it would be ideal to collect cuttings from wild populations, until the mechanisms that drive rooting success for koa are better understood, this could introduce variability that could hinder the progress of testing the efficacy of other factors that are easier to control such as propagation environment, rooting hormone concentration, and stem position.

CHAPTER 2. ROOTING SUCCESS OF ACACIA KOA STEM CUTTINGS FROM MANAGED STOCK PLANTS: DIFFERENCES IN STEM POSITION AND AUXIN APPLICATION METHODS.

Background

Acacia koa (koa) is an endemic Hawaiian tree species that dominates the islands' forested landscape and is an integral component of native plant and animal communities (Baker et al 2009). A majority of koa forests have been lost due to land conversion for crops and cattle, introduction of invasive species, and other environmental pressures (Cuddihy and Stone, 1990). A turn towards more sustainable management practices of this producing this species to reforest its native range and for timber production purposes. Managing for multiple objectives requires selecting attributes that are adventitious to management goals, and begins with identifying superior trees and capturing their genetics through propagation by seed or through the use of cloning vegetative material. Seeds pose a problem, especially with koa, because they are polyploid (4n sets of chromosomes) and are speculated to be self incompatible because pollen is released as early as 5 days sooner than when the flowers are capable of receiving it ((Brewbaker, 1977).). Consequently, offspring from the same tree genetically diverse (Atchinson 1948; Baker et al 2009). A way to overcome effects of genetic recombination is to make a genetic copy of the desirable tree through cloning. Clonal propagation has the advantage of creating uniform genetic material. However, improving these methods for a difficult to root species can be complicated due to the many factors that influence adventitious root formation. These include source and health of stock plants, cutting attributes, post-severance treatment, and propagation environment. This study looks at stem position and the application method for rooting hormone (Leakey 2004).

Within shoot factors change along a gradient that exists from the top of the stem to the bottom affects morphological and physiological characteristics (Leakey, 2004). For example, shoots become more lignified the farther away from the growing tip. The newly formed tissue from the upper part of the shoot may be more prone to form adventitious roots from lower parts of the shoot that are more differentiated and ontogenically older in which the cylinder of schlerychma tissue may present a barrier to root formation (Poupard et al, 1994).

In addition, the phytohormone, auxin, that aids in the production of adventitious roots, is in greater quantities towards the stem tip (Hartmann and Kester, 2010). Therefore, the position of the cuttings in the stem prior to severing from the stock plant can impact its ability to root (Wong et al, 1991). Cuttings located closer to the tip are closer to the main source of auxin biosynthesis in the plant (Hartmann and Kester, 2010). Previous work suggests that basal stem cuttings of African Mahogany had a greater rooting percentage across treatments and clones than those collected from apical nodes (Tchoundjeu and Leakey, 1995). Conversely, Poupard (1994) found that the greatest percentage came from the second position in stems of *Acacia mangium* with the lowest rooting percentage occurring in cutting taken from the base of the stem.

Another factor to be explored in this study is the application method of auxin, a synthetic hormone that is used in clonal propagation to induce adventitious roots. There are different auxin application methods (i.e. liquid, gel, or powder) and each one has a varying degrees of efficacy based on the species and type of cutting material (Cerveney and Gibson, 2005). For koa, Skolmen (1978) found that 3000 ppm of IBA worked best for rooting stem cuttings. Also tested in the Skolmen study was a higher concentration of 8000 ppm of IBA and 1000 ppm of IBA, all applied in powder form. The greatest success came from cuttings rooted with the 3000ppm talc powder.

The overall objective of this study is to determine if the stem position of the cutting as well as how the auxin application method play a role in the successful rooting of stem cuttings for koa collected from wild populations.

Materials and Methods

Seeds were collected from parent trees at the Umikoa Ranch Koa Reforestation Area, (19°55'35.36"N155°20'58.35"W1,580) Hawai'i Island and were stored in the refrigerator until use scarification in mid April 2015. Mechanical abrasion was performed using sand paper to expose the embryo and then wrapped in moist paper towels for 24 hours allowing them to swell with water. After 3-7 days, they were sown directly into D40 pots filled with Promix BX and randomly assigned to a tray. The 107 stock plants were supplied with nutrients and watered through a subirrigation method that used a weak nutrient solution to the plants every time the tray weight dipped below a specific threshold. Watering frequency was determined by the weight of the planting trays and was required to stay at least 75% of field capacity (Appendix B). The nutrient solution used was dependent on the developmental stage of the stock plant and were adapted from Landis et al 1989. After the first set of true leaves emerged an establishment phase regime which consisted of a fertigation solution containing a 40:15:25 (ppm of NPK).This regime was changed after three weeks to an establishment phase regime which consisted of a solution ration of 150:63:100 (ppm of NPK).

Seedlings were 9 weeks old when cuttings were collected. Two cuttings were taken from each stock plant, and "apical" cutting and a "basal" cutting depending on the position within the stem. These two cuttings were the first two that could be taken from the stock plant. The apical meristem was removed and the top three nodes were for the apical cutting and the subsequent three nodes were for the basal cutting. Cuttings were not taken from any lateral stems that

occurred below the leader. Each stock plant was randomly assigned a hormone application type, either Clonex gel 0.3% IBA and >98% water (3,000 mg/L of IBA) or the recommended 3000 mg/L solution of Dip n Grow (1% IBA, 0.5% NAA) to 80% water 2,000 mg/L of IBA and 1,000 mg/L of NAA). After being coated in rooting hormone, cuttings were inserted into a rock wool cube and placed in one of the six enclosed humidity domes. Stock plants were randomized amongst humidity domes, and both cuttings from a stock plant were enclosed in that assigned dome. Each dome had 36 cuttings spaced every other rock wool cell apart. The humidity domes were all placed inside of a growth chamber with suggested light (275 $\mu mom^{-2}s^{-1}$) temperature (24C-26C) humidity (92-98%) and photoperiod (12hrs on, 12 hrs off) settings. Cuttings were checked at least once a day and their conditions and corresponding date were recorded by making notes such as "defoliated", "wilting", "rooted", "rotten".

Figure 7. Humidity domes located full of koa stem cuttings “stuck” in rock wool in a growth chamber at the University of Hawai'i at Manoa’s Pope Greenhouse.

RESULTS

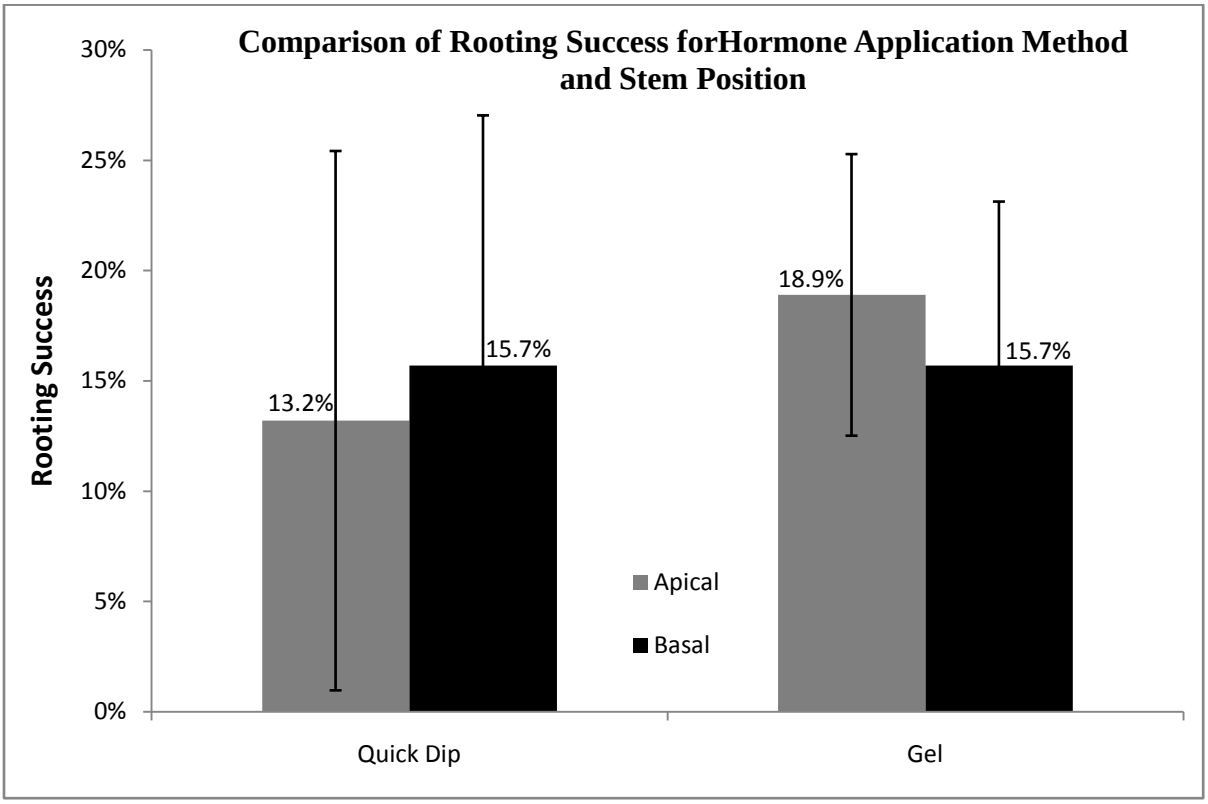

Figure 8. Mean rooting success for the apical and basal stem positions as well as the quick dip and gel rooting hormone application methods (+/-1 standard deviation) (n=206).

The results indicate that the overall mean for the gel method was higher than the quick dip method for the apical position (18.9%, 13.2%) and equal to that of the basal position (15.7%, 15.7%). There was a high amount of variation from the mean as shown in figure 7 by the standard error of the means. The fixed effects of hormone application method and stem position did not yield a significant difference between treatments as seen in table 7 by the observed p-values (Pr(>ChiSq). The only significant effect from this study was the stock plant from which the cutting material came from with an observed p value of 8.98e-10 (Table).

Table 7. Likelihood ratio tests results for each of the factors used the stem position and rooting hormone application method experiment (α=.05)

Likelihood Ratio Test for Fixed and Random Factors					
	Effect Type	*Chi Sq.*	*Df*	*Pr(>ChiSq)*	
Hormone Application Method	Fixed	9 e-04	1	0.9766	
Stem Position	Fixed	1.439	1	2.135	
Block	Random	0	1	1	
Stock Plant ID	Random	37.535	1	8.98E-10	*

DISCUSSION

The experimental factors in this experiment were chosen based on the literature of this study and the prioritization of important influences on rooting success. There is no protocol that can encompass how to promote adventitious rooting in all species and varieties requires optimization for each. Systematically testing and eliminating variables that have no significant impact is critical in determining how to increase rooting success. This study indicates that the rooting hormone application method and stem position did not have any significant impact on the rooting success of stem cuttings causing the rejection of the hypotheses that different treatments would cause a significant improvement. In a previous study performed by Skolmen (1977) rooting hormone application method displayed significant differences between the various methods tested which included: powder, a dilute soak, or a medium that had the synthetic hormone incorporated into it previous to sticking. The powder had the greatest success, but this was most likely due to this method being used on significantly more cuttings. A method that hadn't been tried yet was using a gel form of auxin. For this study, it was hypothesized that this method would have greater success than the 3000 mg/L Dip N' Grow solution because it could adhere to the cutting end and remain in contact with it for a longer amount of time. However, this was not the case and the results from this study suggest that there were no significant differences between the application methods.

The other experimental factor that was hypothesized to have an impact on rooting success was stem position which also yielded no significant difference between treatments. This may have been due to the small number (n=2) amount of cuttings that were taken per stock plant. If more cuttings were taken per plant, it would be a better indication of how the physiological differences changed along the shoot.

This experiment also used nutrient management for stock plants as a bolster for rooting success. This was not included as a test variable, but is notable in its own right. This helps eliminate other factors that could have been unknowingly interfering with rootability that are caused by nutrient deficiencies or toxicities. Stock plant management is an important part of clonal propagation because stem cuttings are stressed when removed from the stock plant and being anatomically and physiologically able to manage that stress depends on the nutrient reserves and ability to still perform physiological processes such as transpiration and photosynthesis.

The only factor that showed to have a significant effect on rooting success was the stock plant in which the cutting was taken from. All stock plants were treated equally during the 9 weeks they grown prior to taking cuttings. Trays were frequently randomized on the benches through this time and all were given the same fertigation treatments. The differences between plants suggest that rooting ability may be genetically linked. Stock plants were propagated from seeds collected in the same area, but from different trees (how many different trees is unknown). The notion that rootability is a heritable characteristic should be further explored and the selection of koa families based on their ability to root should be considered when selecting superior stock.

Figure 9. Successfully rooted basal stem cutting treated with a 3000 mg/L solution of Dip n' Grow.

DISCUSSION AND CONCLUSION

Comparison of Experiments

There were major differences in how the experiments were conducted between chapter 1 and 2. The latter was a refinement of the practices used in previous experiments and aimed to stabilize and control more factors that could influence the rooting ability from pre-severance to post-severance. These included: the propagation environment set-up and conditions, the source of cutting material, the nutritional content of stock plants, and morphological cuttings of the stems were different between the experiments conducted in Hilo versus Honolulu. The overall greater success at the latter location may point to some factors that should be explored to increase the rooting success of koa stem cuttings. However, too many factors were different in order to be able to statistically compare the experiments to one another.

Differences in Source Material

The aim of exploring different factors that influence rooting success is to increase the rooting ability across all genotypes and stock plant material allowing propagators to capture desirable characteristics from wild populations and managed stock plants. Therefore, when collecting for these experiments, there was a great deal of value in trying to root cuttings collected from root suckers in the field. However, other work has show that conditions in the field are more stressful than those found in a nursery setting, especially in respects to sun exposure, water supply, and nutrient availability (Saya et al, 2008). For this reason, stem cuttings taken from a variety of species using “intensively managed container-grown stock plants” have been shown to have a greater physiological ability to form adventitious roots than those collected from the field (Monteuuis et al, 1995).These results supported those found in these studies. The stem cuttings taken from managed stocks plants on Oahu had a greater rooting percentage than

those taken from wild populations on Big Island. Although all of the stem cuttings from wild populations were from plants in the field that appeared very healthy, the stock plants on Oahu were supplied with an optimally watering and nutrient regime based on recommendations by Landis,1989. Statistically significant differences between the stock plants indicated that genetics may play a critical role in rooting success of cuttings. In order to further the progress of tree improvement programs for koa, the results of this study suggest that it is better to select material from managed stock plants and to screen seed stock based on rootability of cuttings.

Differences in Propagation Area

Another major difference between the cuttings collected on Big Island vs. Oahu was the propagation environment for the cuttings. Another objective was to be able to create a protocol that could be used by propagators at a low cost and with minimal technologies. The greenhouse at the Komohana Research Station was a large hoop house covered by polyethylene sheeting and enclosed at either end with a screen. Although this helped promote more moderate temperature and kept pests out, it was very difficult to have exact control over the environmental factors in the propagation area. Not only was the temperature affected by the structure itself, but also local weather patterns. The misting frequency was adjusted to a set time that was optimized to provide moderate temperatures and high humidity to the shade boxes on hot and sunny days. However, on cool and rainy days, this could have caused the cuttings to become oversaturated make them more susceptible to stem rot from anoxic conditions and fungus.

After observing the challenges of trying to propagate under mist in an open air greenhouse, the propagation environment was improved for the experiment in Chapter 3 that was conducted in the Pope Greenhouse at the University of Hawai'i at Manoa on Oahu. This

experiment employed the use of humidity domes inside of a growth chamber in which all of the environmental conditions could be manually controlled

Future Research

As previously mentioned, there are so many different factors that contribute to the successful rooting of stem cuttings and the experiments conducted in these studies only skimmed the surface of work that could be done (Table 2). Future work should systematically explore factors in a way that minimizes that influence of interactive effects between multiple factors. For example, it may benefit to use the same propagation environment in conjunction with the same rooting hormone solution, but different stem positions to better understand how each factor fundamentally effects rooting ability.

Also, future studies should mimic the conditions found in chapter 3 as closely as possible. This experiment used stock plants that were as healthy as possible and were grown in an environment that was controllable and stable. This allowed more certainty in the results because extraneous factors such as weather or unknown health of stock material to be introduced as sources of variation in the results.

Conclusions

Although no significant differences among shade treatments, hormone concentration treatments, and stem position treatments were found, these factors should be further explored by isolating them from confounding effects. Ideally, more levels of each factor would be tested in a more controlled propagation environment to determine the direct effects that the various levels of the treatment have on rootability. Systematically exploring all of the factors that could influence rooting success will require a lot more work, however will be essential to discovering how to clonally propagate and perpetuate desirable characteristics. As land management becomes more

of a focus in the Hawaiian islands, being able propagate trees to meet certain management objectives will be essential for meeting the demands of public and private stakeholders. More importantly, being able to propagate superior stock that have a greater tolerance to introduced anthropogenic environmental pressures will be necessary in securing the future of this beautiful tree in its native landscape.

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APPENDICES:

A. Rooted Cuttings Protocol:

Removing material from stock plants should be conducted in a standardized manner as to not introduce any other variables into rooting studies. Below is a protocol that was adapted from Hawai'i Agricultural Research methods in addition to the *Tropical Trees: Propagation and Planting Manual. Volume 1* (Longman 1993).

i. *Materials and Supplies*

- Snips
- 2 Buckets
- 1 Small container for rooting hormone
- Spray bottle
- Rooting Hormone (Recommended: Dip n' Grow)
- Plant Disinfectant (Recommended: ZeroTol Algaecide/ Bacteriacide/ Fungicide)
- Rooting Media (Recommended: 7500 mg/L or 10,000 mg/L ratio of fine vermiculite to peat moss)
- Dibble Tubes and Racks
- Labels

ii. *Pre-severance:*

Prepare the propagation bench the day before. This includes the following:

- Sanitize are by cleaning propagation area with bleach solution.
- Monitor greenhouse conditions such as light (PAR), humidity, and temperature.
- Install and check irrigation system including water output and the duration and frequency of the misting regime.
- Preparing the rooting media by filling containers with desirable mix and allowing to become saturated 24 hours prior to taking rooted cuttings
- Create holes in the media by inserting a small stick
- Ensure that stock plants are healthy through visually checking for healthy leaf characteristics and the absence of pest or pathogen damage.

For stock plants that are rooted in the ground, prepare a bucket of water to place all severed material in for transportation to the propagation bench.

For container grown stock plants, move as close to the propagation bench as possible.

**It is imperative to keep cuttings moist throughout the entire process, do not leave severed material on bench. Either place in water, a ziploc bag with a wet paper towel, or insert directly into rooting media.

Prepare a 3000 mg/L solution of Dip n' Grow and a 1:800 solution of ZeroTol. (Approximately 20mL/gallon)

iii. *Severance:*
Remove up to 7500 mg/L of the leaf area depending on the size of the leaf. Longman recommends keeping 50cm^2.
Remove the terminal bud and any leaves that are in poor conditions.
Two-node cuttings will be taken. All cuts should be made at a 45 degree angle above the first node of the cuttings below. The stem of the cuttings should be no more than 15cm in length.
Place freshly severed material in beaker while removing additional cuttings to the stem
Briefly submerge in ZeroTol and let cuttings drain on bench for no longer than 10 minutes.
Submerge in clean water to remove excess ZeroTol
Dip basal portion of cutting in Dip N' Grow
Place cutting in dibble tube, gently packing media around it.
Create a label containing information such as family, individual, and date.
Mist with spray bottle after insertion.

vi. *Post Severance:*
Continue monitoring greenhouse conditions
Check cuttings for:
saturation of media
retention and health of leaves (remove material that has fallen)
pest damage
Adjust watering and shade levels as needed
After 4 weeks, check cuttings for roots. If they have not produced roots, but still have healthy leaves allow additional two weeks for rooting.

v. *Other Considerations:*
Monitor misting regime to ensure that cuttings are receiving the optimal amount of water. Too much will cause the rooting media to become saturated and the basal end of the stem to rot. Conversely, too little will cause the leaves to wilt severely lowering photosynthetic capacity resulting in unsuccessful rooting.
Cuttings should be taken early in the morning or on an overcast day. It is imperative that they do not dry out during the process and limited exposure to the sun can be very help reduce the amount of stress experienced by the cutting.
The degree of lignification should be taken into account when choosing material to root. The less lignified, the easier it is for root initials to form. Also, only use material that has true leaves which are easily identified as bipinnately compound as opposed to the mature phyllodes.

B. NUTRIENT MANAGEMENT

Subirrigation

1. Weigh each tray using the scale in the lab and record weights.
2. Determine if the dry down percentage falls between 65%-75% using the "Dry Down Guide" spreadsheet. If the majority of trays fall within this range, then it is time to irrigate.
3. Place trays in black/yellow bins. These bins are filled with a weak nutrient solution, so the plants are being fertilized and watered at the same time.
4. Allow trays to soak in bins for 10-15 minutes.
5. Remove from bins.

Daily:

Check tray weights in the morning (9AM) and afternoon (1PM). Dry down usually takes about 1 day, but can be longer/ shorter depending on the weather.
Enter weight data into the spreadsheet.

I was using two different fertilizer concentrations for the duration of growing the stock plants. The first was considered the "establishment phase" and lasted 3 weeks at which point it is switched to the "rapid-growth phase".

Establishment Phase:

Nutrient ratio: 40ppm N/ 15ppm P/ 25ppm K
.8ml of FloraMicro
.3ml of PKMgS (100g Monopotassium; 71.75 Magnesium Sulfate/ 500mL H20 stock solution)

.8ml of FM + .3ml of PKMgS + 998.9ml of H20=1L of Fertigation Solution

Rapid Growth Phase:

--Nutrient ratio: 150ppm N/ 63ppm P/ 100ppm K
-- 2ml of FloraMicro
--1.4ml of PKMgS
--.65ml of CaN03

2ml FM + 1.4ml PKMgS +.65ml CaN03 + 995.95ml of H20= 1L of Fertigation Solution