

Applied Genetic Conservation of Hawaiian *Acacia koa*: an Eco-Regional Approach¹

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Abstract

Koa (*Acacia koa*) is a valuable tree species economically, ecologically, and culturally in Hawaii. A vascular wilt disease of koa, caused by the fungal pathogen *Fusarium oxysporum* f. sp. *koae* (FOXY), causes high rates of mortality in field plantings and threatens native koa forests in Hawaii. Producing seeds with genetic resistance to FOXY is vital to successful koa reforestation and restoration. The Hawaii Agriculture Research Center (HARC), with both public and private partners, operates a tree improvement program to develop koa wilt resistant populations in Hawaii. The population genetics of koa are poorly understood across the broad range of habits that koa occupies and seed zones have not been sufficiently established. Thus, HARC estimates seed zones based on biogeographic variables and has selected wilt resistant koa populations for six ecological regions (eco-regions) in Hawaii. This conservative approach, based on planting locally sourced germplasm, is often a requirement of many restoration programs in the state. We further consider population genomic (single-nucleotide polymorphism) data in relation to the proposed eco-regions. Preliminary analyses suggest genetic differences among and within islands that are broadly consistent with eco-regions, but also suggest additional population differences that should be considered in genetic conservation of koa.

Koa Significance

Acacia koa (koa) is a highly valuable timber tree species endemic to the Hawaiian Islands. Koa is a dominant canopy tree and keystone species in native forests where it provides critical habitats for endangered native birds and epiphytic plants. Koa is also a nitrogen-fixing tree legume that forms both root and canopy nodules in association with *Bradyrhizobium* (Leary et al. 2004). Under ideal conditions, koa grows to heights of over 30 m and lives several 100 years. This tree is of immense cultural importance to native Hawaiians, as its wood is used for a range of traditional applications. Most notably, it is the preferred wood for construction of traditional Hawaiian voyaging canoes. Koa timber is used for producing musical instruments, specialty furniture, and other high value craft goods. The very limited supply of commercial quality trees is a significant limiting factor to the Hawaiian forestry industry, with the total annual value estimated at \$20 to \$30 million (Yanagida et al. 2004).

Koa Distribution

Owing to its topographic and oceanic island position, the Hawaiian archipelago contains a wide range of terrestrial ecological zones that include alpine, subalpine, montane, lowland, and coastal (Juvik and Juvik 1998). Within each ecological zone, three general moisture regimes are recognized, dry (<1,200 mm), mesic (1,200 to 2,500 mm), and wet (>2,500 mm) of annual rainfall (Mueller-Dombois 1992). As a

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consequence, Hawaii's small land base supports a high level of ecosystem richness and diversity. However, Hawaii's forests are species poor, similar to many remote oceanic islands. In Hawaiian forest ecosystems, koa is a conspicuous component occupying a wide range of environments (Baker et al. 2009). Noted for its environmental plasticity, koa occurs in subalpine, montane, wet, and lowland forest ecozones across the four main islands (Hawaii, Maui, Oahu, and Kauai) (fig. 1).

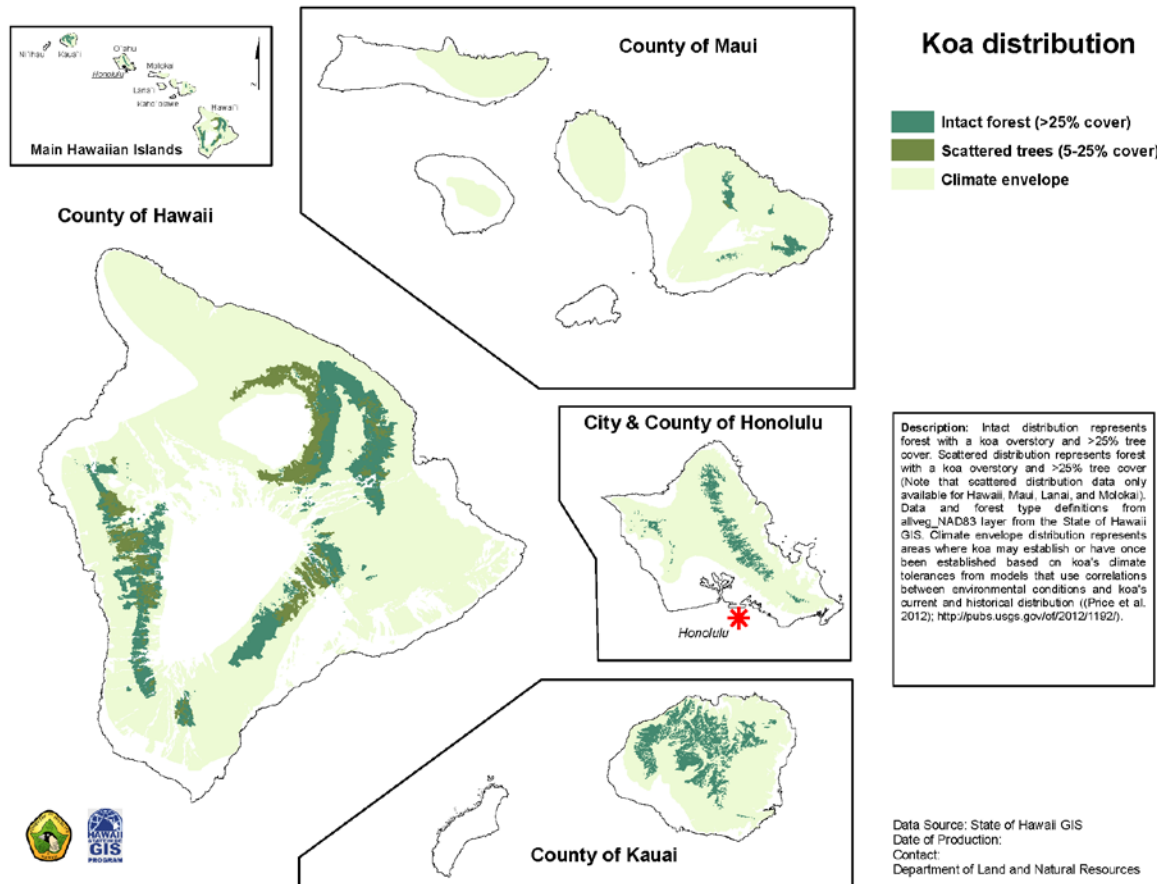


Figure 1—Current koa concentrations in the Hawaiian Islands (Price et al. 2012).

Koa Wilt Disease

Koa wilt disease is a vascular wilt caused by the fungal pathogen *Fusarium oxysporum* f. sp. *koae*. This soil pathogen invades susceptible plants through the root system, enters the xylem and restricts water transport, eventually leading to tree mortality. In Hawaii's remaining koa forests, koa wilt disease has caused further decline and is considered one of the greatest threats to the resource (Anderson and Gardner 1998, Anderson et al. 2002, Dudley et al. 2007, Gardner 1980, James 2005). Koa wilt disease severely restricts koa reforestation in most low to mid-elevation locations (sea level to approximately 1,000 m elevation) with mortality rates commonly exceeding 75 percent. As low elevation sites provide the greatest growth potential for this tropical species, there is significant incentive to develop wilt resistant populations for commercial reforestation.

The virulence of *Fusarium oxysporum* in relationship to soil temperature is well studied in many host species, with increased virulence at higher temperatures (Clayton 1923, Landa et al. 2006, Scott et al.

2001). While these temperature effects have not been studied directly in koa, the cooler temperatures at higher elevations may explain the current lower disease severity found at these locations. However, global climate change and the subsequent increase in soil temperature threatens to increase the disease severity in areas where it is currently limited. Developing and conserving wilt resistant high elevation seed sources will provide a level of biosecurity to mitigate the predicted effects of increased soil temperatures and ensure that koa remains a keystone species in Hawaii's remaining native forests.

Koa Genetics

Koa is primarily a cross-pollinating species, or outcrossing (i.e., mating among unrelated individuals) species, which is important to maintain genetic diversity within populations. Unlike most *Acacia* spp., koa is a tetraploid species and contains four sets of chromosomes within the cell nucleus, facilitating high levels of genetic variability (Atchinson 1948, Carr 1978, Le Roux et al. 2014, Shi 2003). Koa's polyploidy complicates analysis of genetic studies, and the literature conflicts regarding koa's tetraploid origins. Most previous literature claims koa to be an allotetraploid with disomic segregation and genetic studies were analyzed based on this assumption (Adamski et al. 2012, Brewbaker 1977, Shi 2003, Sun 1996). Nevertheless, no strong evidence supports this claim. Recent work provides evidence to support that koa is indeed an autotetraploid, derived from *A. melanoxylon*, with the species diverging over 4.9 million years ago (Brown et al. 2012, Le Roux et al. 2014).

Variation

A large degree of phenotypic variation has been documented as early as the 19th century and has led to varying taxonomic classifications (Adamski et al. 2012, Daehler et al. 1999, Hillebrand 1888, St. John 1979, Sun 1996, Wagner 1990). The most commonly described variations occur in: phyllode shape, pod shape, seed shape and arrangement, inflorescence and flower structure, growth form and rates, wood characteristics, and disease resistance (fig. 2). Isozyme study on populations from across Hawaii revealed a large degree of diversity (Conkle 1997). The variable genes studied had between three to seven alleles and an average expected heterozygosity of 0.41. The study utilized the variable genes to calculate genetic distance and found Hawaii Island populations were distinct from Kauai, Oahu and Maui populations. Recent research confirmed a large degree of genetic variation in koa, but found no correlation between genetic and geographic distance (Adamski et al. 2012), and 73 percent of genetic variation was partitioned among individuals within a population, compared with 6.5 percent for the whole taxa and 21 percent among populations. Several common garden trials examine the relationship between genetic and phenotypic variation were planted during the 1990s as a joint effort between the University of Hawaii and HARC (Daehler et al. 1999, Shi 2003, Sun 1996). While these trials had high rates of mortality (>70 percent) from koa wilt disease, several key observations were made: estimated family heritability for height and diameter at breast height (1.37 m) approached 0.75; genetic variations were observed for disease resistance; tree form, seed size, seed shape, seed weight, seedling growth, juvenile growth, phyllode development, phyllode shape, nectary, flowering pattern and duration of vegetative stage; and Oahu and Hawaii Island trials showed a significant genotype by environment interaction.



Figure 2—Phenotypic differences in pods, seeds, and phyllodes. (D. Adamski)

The multiple uses of koa justify a robust genetic conservation program sensitive to the needs of the various stakeholders while reversing the genetic degradation of this iconic species. Koa's apparent high degree of genetic and morphological variation makes it a strong candidate for genetic improvement. Beyond commercial forestry, koa is a keystone species in Hawaiian native forests and koa forest restoration is a primary objective for numerous local conservation and community groups.

Establishing Seed Zones: an Eco-Regional Approach

Sustainable forestry emphasizing restoration or reforestation requires choosing the proper seed source of high genetic quality. It is critical that the seed be ecologically well adapted, productive, and healthy (Morgenstern 1996), and this will have a significant impact on the planted forest. Knowledge about the geographic variation of any species aids in selection of the most appropriate provenance for reforestation and restoration (White et al. 2007, Wright 1976, Zobel and Talbert 1984).

Accordingly, many landowners/managers and restoration groups are reluctant to plant koa originating from outside their eco-region. The current recommendation is to plant locally sourced seed to ensure that seedlings will be well adapted to site conditions (Baker et al. 2009). This is because seed or planting zones (a seed zone is a single geographical or ecological unit within the range of the species based on ecological and genetic criteria) for koa in Hawaii are not well defined, due in part to the limited information on koa population genetics. Planting seedlings from seed collected and planted within the same established ecological zone would be considered a “local” source of seed (White et al 2007).

The HARC—in collaboration with the U.S. Department of Agriculture Forest Service, the State of Hawaii, Department of Land and Natural Resources, and County of Maui, Department of Water Supply—has an on-going program to identify and conserve *Fusarium* wilt resistant koa populations. The lack of established koa seed zones necessitated utilizing the best currently available information to estimate seed zones and to develop wilt resistant populations from those preliminary zones. This preliminary demarcation and delineation of seed zones for koa is based on ecological data (Morgenstern 1996).

Landscape classification variables or zonation can be an efficient means for quantification of biological diversity (Ferrier and Smith 1990). Our goal is to provide a framework to guide genetic conservation and more precisely deploy koa seed sources for restoration and reforestation efforts. This is a conservative approach seeking to ensure that planted trees are well adapted to local conditions and similar genetically to local populations with the objective of dynamic genetic conservation of fragmented koa populations at low and mid-elevation sites across the state (Campbell 1975, Dudley et al. 2015).

Basis for Seed Zones

At the outset of this project, owing to the impact of Hawaii's complex biogeography on koa population structure, we proposed the following framework to utilize (the currently best available) geographic and genetic variation data to delineate preliminary/provisional koa seed zones (Hamann et al. 2005, White et al. 2007, Ying and Yanchuk 2006). This is a synthesis of geographic, climatic and vegetation patterns, we describe as geo-climatic, or ecological regions (eco-regions) (Pojar et al. 1987). HARC's preliminary eco-regions for koa populations are delineated as follows:

- By island, discontinuous by island and within island.
- By aspect within island, primarily windward (wet) and leeward (dry) zones.
- By elevational sub-zone within each zone: sea level to 600 m (low); 600 m to 1200 m (mid); 1200 m to 1800 m (high).
- By special situation (*A. koa*'ia in Kohala, Hawaii; low-elevation *A. koa*, west Maui).

The lack of more robust genetic data, the multiple uses for koa, and the ecological and cultural significance force a conservative approach to genetic conservation and seed zone delineation. Therefore, the HARC koa improvement program is primarily based on developing *in-situ* seed orchards for local koa populations in the different geographic zones, referred to as eco-regions. This approach allows for increased flexibility once koa population genetics and the relationship between genetic variation and adaptability to Hawaii's numerous ecological zones are better understood. Thus, these preliminary seed zones are expected to evolve over time with increases in knowledge of genetics and ecology of koa, and as new analytical approaches arise. This will assist in further elucidation and delineation of these seed zones.

Refining Koa Seed Zones: Incorporating Genomic Data

To better understand these preliminary proposed seed zones for koa in relation to genetic variation, we analyzed genomic data consisting of 11,002 diallelic single-nucleotide polymorphisms (SNPs) derived from genotyping by sequencing (GBS; Elshire et al. 2011) for 311 individual *A. koa* trees collected across Hawaii as part of an ongoing study (Gugger, Liang and Wright, unpublished data). We used SNP allele frequencies to calculate pair-wise F_{ST} values among eco-regions modified according to observed population genetic structure (Wright 1965). Although it is problematic to calculate F_{ST} in tetraploid populations using SNP data that were coerced to be "diploid" because assumptions can about allele frequency calculations are not necessarily met, our goal is to compare the relative differences among islands and proposed seed zones.

For the eco-region seed zones, we compared trees from Kauai that are understood to be from the native population of the island with Kauai trees that potentially originated from other islands as a result of reforestation efforts. On Maui, we compared two windward sources from opposite ends of the windward zone (see fig. 3 showing the seed zones). On Oahu, we did not have enough samples to compare the Koolau and Waianae (two different mountain ranges) sources, and only show data for the Koolau population. On Hawaii Island, four eco-region seed zones were proposed (fig. 3); however, we can only compare three, as we do not have enough sources from the *A. koa*ia zone.

Figure 5 shows a dendrogram of hierarchical clustering based on Euclidean distance of pairwise F_{ST} among eco-regions. Eco-regions connected closer together on the tree have more similar pairwise F_{ST} values, suggesting they are more similar genetically. The results suggest genetic differences among all

four islands, with comparisons between the most geographically distant islands (Kauai and Hawaii) showing the highest levels of differentiation. Within islands, differences were much lower, with nearly no difference between the native and potentially introduced trees on Kauai. The two Maui seed zones showed the greatest differences, while there were smaller differences among the Hawaii seed zones.

As an exploratory analysis of genetic structure in relation to eco-regions, we modified the ecological seed zones in Maui and Hawaii to genetic seed zones (fig. 4, 6). To preliminarily define genetic seed zones, genetic clusters were estimated with Admixture 1.3 (Alexander et al. 2009) using the SNP data. Individuals from Maui and Hawaii were generally assigned to the cluster from which they derived their largest ancestry (highest Q). We recalculated the F_{ST} values using these genomic seed zones, and found a higher level of differentiation within those two islands (fig. 6) in comparison to the zones based purely on eco-region. Interestingly, the new Maui Low elevation-wet seed zone is very similar to the Oahu Koolau seed zone, with little differentiation between them (less than between the two Maui seed zones; low elevation trees from Maui are more similar to trees on Oahu than to high elevation trees on Maui).

The genetic seed zones presented here are preliminary only, and further analysis is needed to better define the zones. However, our goal with these alternative genomic zones is to show that there is genetic differentiation within island populations of *A. koa*, particularly on Hawaii Island, and to suggest that further refinements of the proposed seed zones may be necessary to account for these genetic differences. Indeed, the origin of koa trees within islands appears to be associated with genetic differentiation.

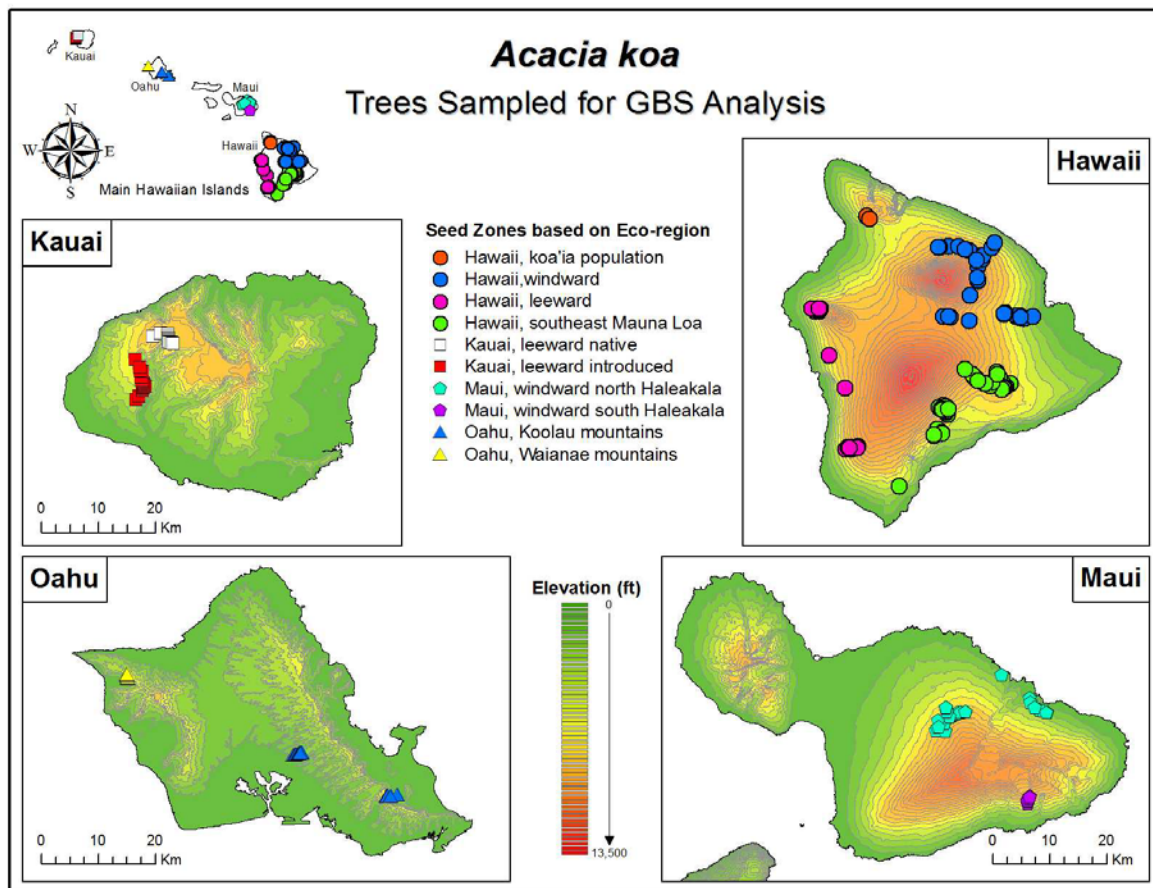


Figure 3—Seeds zones of 311 sample trees used in genomic analyses based on eco-region.

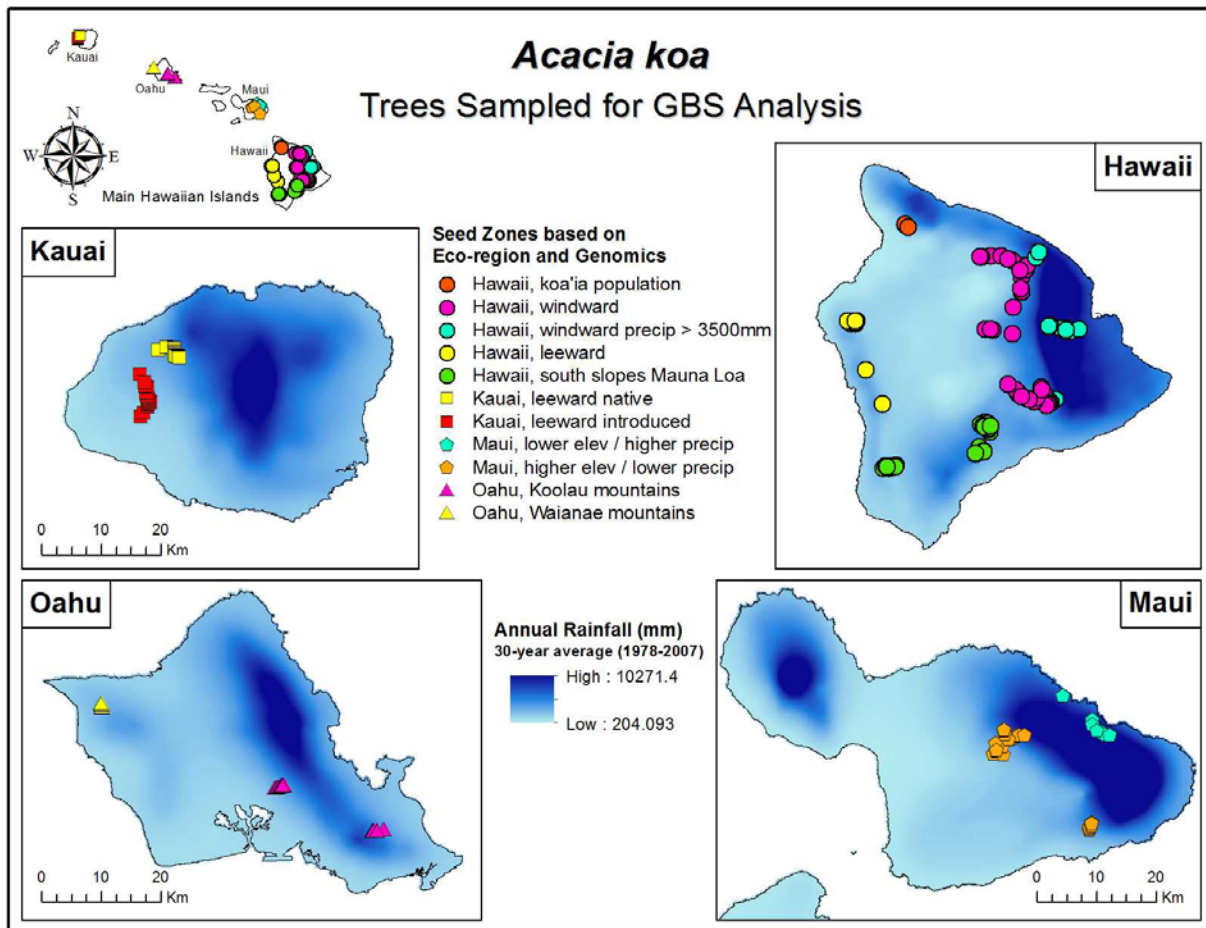


Figure 4—Seed zones of 311 samples used in genomic analyses based on a combination of eco-region and genomic data.

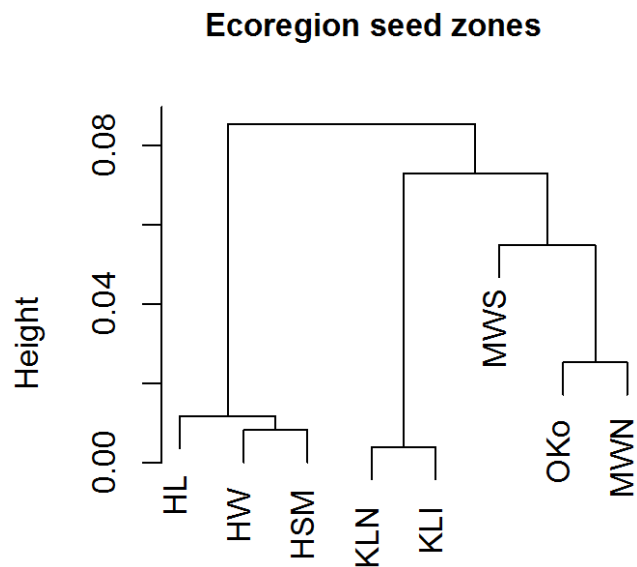


Figure 5—Hierarchical clustering based on Euclidean distance of pairwise F_{ST} among eco-regions, as implemented in R 3.3.0. HL- Hawaii Leeward, HW- Hawaii Windward, HSM- Hawaii South Slope of Mauna Loa, KLN- Kauai Leeward Native, KLI- Kauai Leeward Introduced, MWS- Maui Windward South, OKo- Oahu Koolau, MWN- Maui Windward North.

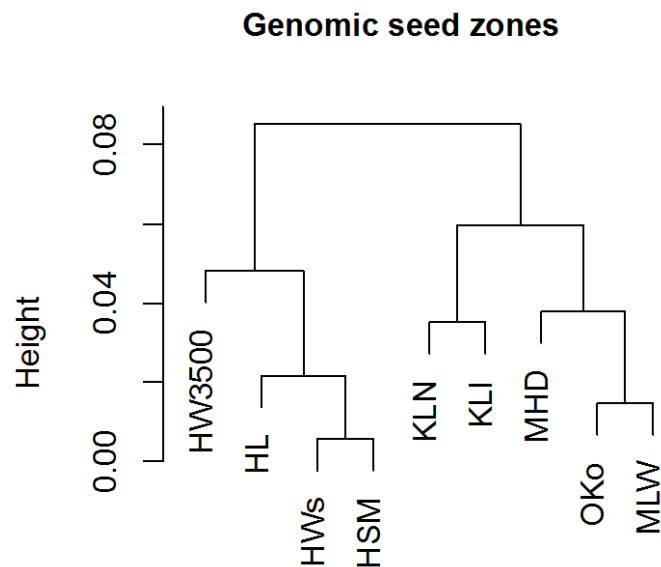


Figure 6—Hierarchical clustering based on Euclidean distance of pairwise F_{ST} among eco-regions modified according to genomic data, as implemented in R 3.3.0. HW3500- Hawaii Windward, annual precipitation >3500 mm, HL- Hawaii Leeward, Hawaii Windward Slopes, HSM- Hawaii South Slope of Mauna Loa, KLN- Kauai Leeward Native, KLI- Kauai Leeward Introduced, MHD- Maui Higher Drier, OKo- Oahu Koolau, MLW- Maui Lower Wetter.

Application of Seed Zones to HARC Fusarium Wilt Resistant Koa Wilt Resistance Screening

Koa wilt disease is caused by a pathogen of unknown origin found throughout Hawaii and is a major impediment to successful koa reforestation and restoration. HARC utilizes an artificial inoculation test to select half-sib families with an increased frequency of genetic resistance to the causal agent, *F. oxysporum* f. sp. *koae* (Dudley 2013, 2015). Highly virulent isolates are used to inoculate very young koa seedlings from half-sib families. Family survival ranges from 0 percent in the most susceptible to over 80 percent in the most resistant, with an average survival of approximately 40 percent. This rapid and reliable screening method enables HARC to quickly screen koa families collected from various eco-regions across the state. Wilt resistant families are then out-planted in field trials/seed orchards located within the mother tree's originating eco-regions to monitor the durability of resistance, produce wilt resistant seed and maintain *in-situ* genetic conservation (fig. 7).

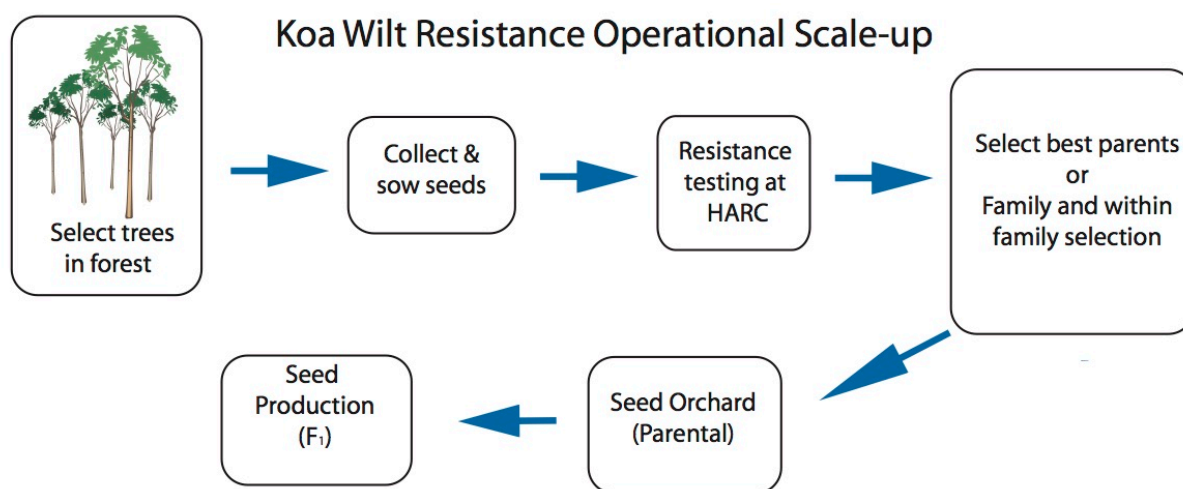


Figure 7—*Acacia koa* wilt resistance tree improvement (1st cycle).

Seed Collection Methods

Koa seeds are collected from individual dominant, or co-dominant mother trees with vigorous canopies within specific eco-regions. These candidate trees are identified by collection location and geo-referenced. Care is taken to avoid collecting from isolated trees with potentially elevated levels of selfed offspring. This permits the capture and study of genetic variation within and between populations (Willan 1985). Following seed collection, a database was developed from seed collection data.

HARC's standard practice is to collect a target population size of at least 50 to 100 mother trees per eco-region (Nikles 1974). Ideally, samples trees are at least 50 m apart to avoid relatedness among seed lots from different mother trees, and equal amounts of seed are collected from different quadrants of the canopy. The goal is for open-pollinated offspring from each selected mother tree, to be the result of numerous male pollen parents. Approximately 500 viable seeds are collected from each mother tree for wilt resistance screening and genetic conservation effort. Koa seed is a non-recalcitrant with a hard outer shell, permitting seed to be stored for decades under proper conditions.

In-situ Seed Orchards

Since 2011, koa populations from five eco-regions have been screened for wilt resistance; Koolau Mountain (Oahu), Southeast Mauna Loa (Hawaii Island), windward Hawaii Island, windward Haleakala (Maui), and Kokee (Kauai) (table 1). A leeward Haleakala (Maui) population is currently scheduled for

screening by the end of 2016, but samples were not collected from this region for the genomic portion of this project.

Field sites were selected for planting wilt resistant families selected from greenhouse screening trials. In 2012 to 2016, HARC planted seed orchards for the Koolau, Southeast Mauna Loa and windward Haleakala eco-regions. Wilt resistant families have been selected for the windward Hawaii Island and Kokee eco-regions and seed orchard establishment is scheduled for late 2016 to 2017. Early survival data indicates a significant improvement over control families, particularly at the Oahu site, where pathogen pressure is highest. Continued monitoring and data collection are critical to understand the durability of resistance. If survival remains high, thinning will be based primarily on growth characteristics such as stem form and volume. It is anticipated seed production will commence is 3 to 5 years after outplanting.

Table 1—HARC Fusarium wilt resistant seed orchards

Eco-region	Island	Germplasm number of families	Planting year	Seed zone of mother trees ^a (GBS)
Southeast Mauna Loa	Hawaii	12 families	2012	HSM & HW.s
Windward Haleakala	Maui	15 families	2013	M.HL
Koolau	Oahu	34 families	2012	OK
Kokee	Kauai	25 families	2016 ²	KLN
Leeward Haleakala	Maui	~ 25 families	2016 ²	not tested
Windward Hawaii	Hawaii	20 families	2017 ²	HW3500

^a HSM- Hawaii South Slope of Mauna Loa; HW.s- Hawaii Windward Slopes; M.HL - Maui Higher elevation, Lower precipitation; OK- Oahu Koolau; KLN- Kauai Leeward Native; HW3500- Windward, annual precipitation >3500 mm.

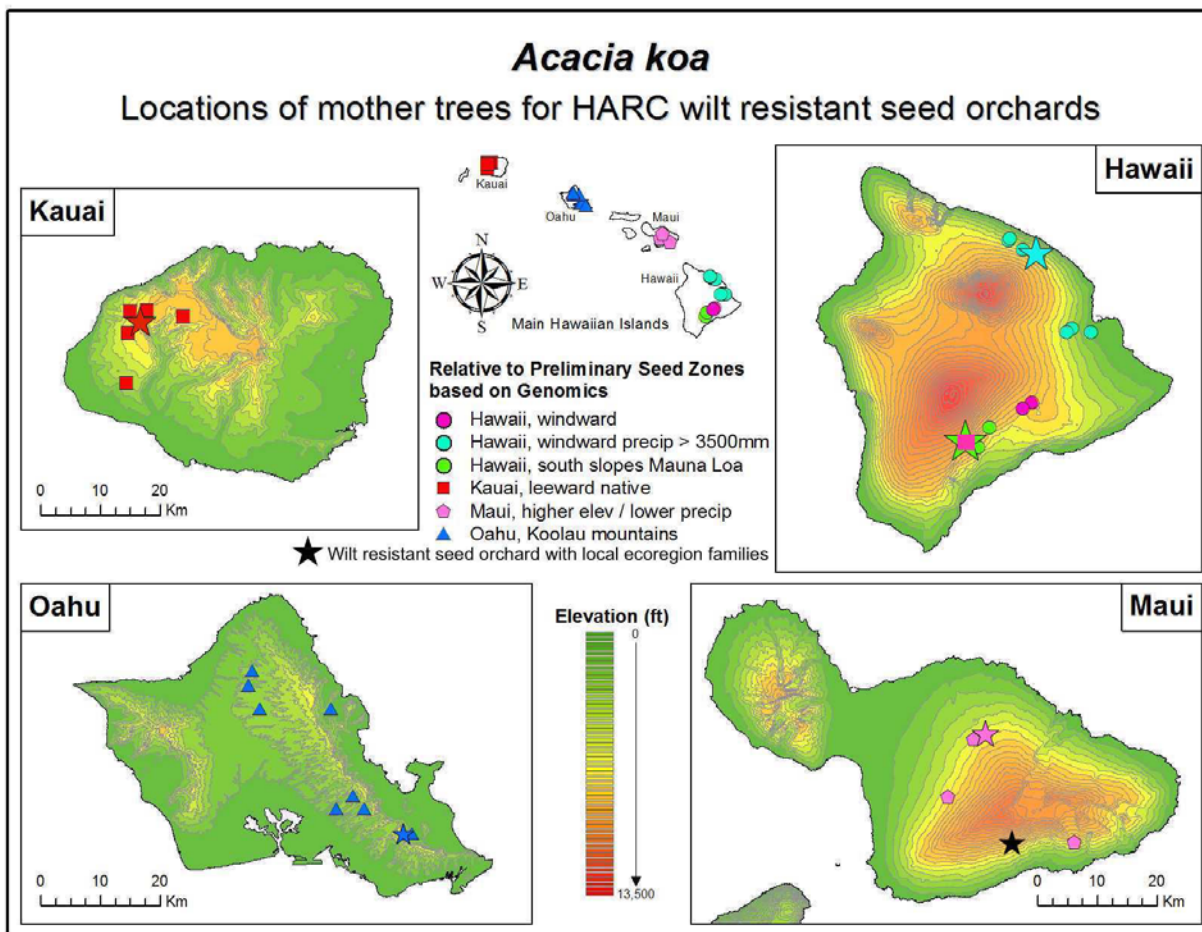


Figure 8—Locations of HARC koa wilt resistant seed orchards and mother trees. Color of icons indicates from which preliminary seed zone the mother trees originate based on available genomic data.

The location of the selected mother trees from which the seedlings included in the seed orchards was plotted and compared to the preliminary seed zones based on genomic data (table 1 and fig. 8). The eco-region approach to seed zone delineation is relatively consistent with the preliminary genomic delineations. All orchards exclusively contain trees from the local zone, with the exception of the South Mauna Loa orchard, which utilized mother tree from both the local, and the Hawaii windward zone. Overall, genomic data suggests that the eco-region approach to seed zone delineation was appropriate and should continue, as genomic-based approaches are refined.

Summary and Discussion

Developing koa planting stock that is resistant to the wilt-causing fungus *F. oxysporum* is a critical step to meet the overall objectives of conserving Hawaii's remaining koa forests, restoring koa to its native range and ensuring a sustainable supply of koa timber for future generations. It would significantly reduce risk and uncertainty associated with growing koa in plantations below approximately 1,000 m in elevation. The rapid disease screening methods developed during the early phases of this project gives koa improvement programs the ability to accomplish this goal in a timely and efficient manner. It should be noted that some continuing effort is always needed to obtain new, virulent strains of *F. oxysporum* and to maintain virulent strains for use in these screening operations.

Koa's significance to the ecology, economy and culture of Hawaii mandate conserving wilt resistant koa populations while maintaining genetic differentiation within the species. Therefore, the HARC koa

improvement program is primarily based on developing disease resistant koa seedling seed orchards for local koa populations in the different eco-regions by island. Other than wilt resistance, these koa families approximate the wide range of genetic variation and diversity found in natural populations. This approach allows for flexibility as koa population genetics and the relationship between genetic variation, population structure and adaptability to Hawaii's numerous ecological zones become better understood.

The opportunity to further refine koa seed zones recently emerged by the characterization of genomic data based on statewide sampling. This has resulted in further refinement and verification of koa seed zones. However, these genetic seed zones are preliminary in nature, and further analysis is needed to refine and better delineate these zones. A landscape genomic analysis is currently underway using the single-nucleotide polymorphism data described here, which raise some interesting questions that merit further investigation. Additional sampling and genomic sequencing will contribute data to address these and other questions, and allow for further refinement of the proposed seed zones.

The goal of establishing wilt resistant koa seed orchards comprised of locally sourced germplasm in numerous ecological regions throughout the state is now underway, with several sites established and three additional sites scheduled to be installed by mid-2017. In the future, additional sites are planned in eco-regions that are currently under represented.

The result of this endeavor will be locally adapted, eco-region specific koa seed that allows for the restoration of this iconic species and commercial reforestation opportunities. It is envisioned this will include further development of a distribution and seed banking network for the release of improved koa seed for non-industrial and forest landowners and managers across the state. This will allow for the efficient distribution of improved (locally adapted, genetically diverse, disease resistant) koa seed, permitting the reduction of risk and increased confidence in future reforestation and restoration efforts.

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