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Applied disease screening and selection program for resistance to vascular wilt in Hawaiian *Acacia koa*§

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***Acacia koa* is a valuable tree species economically, ecologically and culturally in Hawai'i. A vascular wilt disease of *A. koa* resulting from infection by the fungal pathogen *Fusarium oxysporum* f. sp. *koae* (FOXY) causes high rates of mortality in field plantings and threatens native *A. koa* forests in Hawai'i. Landowners are reluctant to consider *A. koa* for reforestation and restoration in many areas due to the threat of FOXY. Producing seeds or propagules with genetic resistance to FOXY is vital to successful *A. koa* reforestation and restoration. Virulent FOXY isolates were used in seedling inoculation trials to evaluate resistance levels among *A. koa* families in greenhouse experiments. Seedling survival varied by family, ranging from 3% to 92%, with an overall average of 46%. One clonal and three seedling field trials were established in 2012 and 2013 using selected families based on the inoculation trials. The greenhouse screening method serves as a powerful tool to rapidly evaluate *A. koa* families prior to outplanting, but the field trial data are needed to further validate the results and to monitor the durability over time. The field trials will also serve as a source of germplasm for selection of other commercial traits.**

Keyword: *Acacia koa*, disease resistance, *Fusarium* wilt, seedling inoculation, tree improvement

Introduction

Acacia koa Gray is a highly valuable timber tree species endemic to the Hawaiian Islands. *Acacia koa* timber is considered a priced commodity, and has a current market value of up to $125 per board foot (Baker et al. 2009). *Acacia koa* wood is used for fine furniture, decorative items, musical instruments and jewellery. It is also the preferred wood for construction of Hawaiian voyaging canoes. Significant *A. koa* forests are found on four of the major Hawaiian islands: Hawai'i, Maui, Oahu and Kauai (Baker et al. 2009; Adamski et al. 2012). The gross value of *A. koa* timber and the wood products produced annually is estimated to be in the range of US$20–30 million (Yanagida et al. 2004). *Acacia koa* is a dominant canopy tree in the native Hawaiian forests where it provides suitable habitats for endangered native birds and epiphytic plants. *Acacia koa* is also a nitrogen-fixing tree legume that forms both root and canopy nodules in association with *Bradyrhizobium* (Leary et al. 2004).

Acacia koa is a cross-pollinating, tetraploid species with $2n = 52$ (Atchinson 1948; Carr 1978; Shi 2003). *Acacia koa* is an autotetraploid, originating from a genome duplication of the Australian species *A. melanoxylon* (Murphy et al. 2010; Brown et al. 2012; Le Roux et al. 2014). While there has been some dispute on the origins of *A. koa*, recent phylogenetic work indicates *A. koa* colonised the Hawaiian Islands long before possible human-mediated transport, with colonisation likely occurring millions of years ago (Le Roux et al. 2014). This view is further supported by the historical pollen record (Hotchkiss and Juvik 1999), and the presence of several endemic insects known to be host-specific on *A. koa* (Gagne 1979). These results also correspond with the phenotypic and genetic diversity described in *A. koa* (Hillebrand 1888; St John 1979; Wagner et al. 1990; Conkle 1996; Sun 1996; Daehler et al. 1999; Adamski et al. 2012). While molecular genetic studies show differentiation between *A. koa* and *A. melanoxylon*, *A. koa and A. heterophylla* are closely related, warranting reclassification as the same species (Le Roux et al. 2014).

The area under native *A. koa* forests in Hawai'i declined significantly due to mass deforestation for agriculture and ranching during the nineteenth and early twentieth centuries. In addition, the presence of koa wilt disease caused by *Fusarium oxysporum* f. sp. *koae* (*FOXY*) has caused further decline in recent years (Gardner 1980; Anderson and Gardner 1998; Anderson et al. 2002; James 2005; Dudley et al. 2007) (Figures 1 and 2). *FOXY*

§This article is based on a paper presented at the 'Sustaining the Future of Acacia Plantation Forestry' IUFRO WP 2.08.07 conference, March 2014, Hue, Vietnam

is primarily a soil-dwelling fungus, which typically invades susceptible plants through the root system (MacHardy and Beckmann 1981). Upon entering the roots, successful infection requires entrance into the plant's xylem tissue, where it spreads upwards and leads to the disruption of water movement and eventual plant death (MacHardy and Beckmann 1981). The exact mechanism the pathogen utilises to disrupt water flow varies by host and is dependent on specific host–pathogen interactions. The nature of these interactions between *A. koa* and *FOXY* is unknown.

There is increasing desire within the forest community of Hawai'i to prevent *A. koa* forests from further decline and to restore the native range of *A. koa*. There is also strong interest in planting *A. koa* as a commercial plantation species on abandoned sugarcane and pineapple lands (Newell and Buck 1996). Brewbaker and his colleagues (Shi and Brewbaker 2004) operated an *A. koa* domestication program since 1990 at the University of Hawai'i at Manoa, but inconsistent funding and the emergence of koa wilt disease have impeded progress. The Hawai'i Agriculture Research Center (HARC) worked closely with Brewbaker's program during the 1990s and experienced similar problems with koa wilt disease.

Koa wilt disease caused high rates of mortality in genetic trials at the HARC Maunawili Experiment Station and at the University of Hawai'i's Hamakua Station during the 1990s (Sun 1996; Daehler and Dudley 2002; Shi 2003). Shi (2003) analysed mortality data from field trials and found variation in survival between half-sib families. The most resistant families had 80% survival, whereas the most susceptible families had 0% survival at year three. Mortality was greatest from age one to six years. The overall mortality at year seven was typically around 75%. Shi (2003) found no correlation between disease resistance and growth rate. The results provide strong evidence of genetic resistance to the disease and indicate that selection of resistant families based on survival rates is appropriate. Shi (2003) also argued that environmental factors complicate selecting for disease resistance and an artificial inoculation technique would be helpful. The high costs associated with field trials and the long time periods needed to select resistant families from these trials provided further incentive to develop artificial inoculation methods capable of distinguishing high surviving families. In 2003, the US Department of Agriculture (USDA) Forest Service and HARC held discussions to outline and begin a program to develop populations of *A. koa* resistant to *FOXY* (Sniezko 2003). Trials to establish protocols for artificial inoculation of seedlings and screening for resistance began in 2004. Components of a viable program included developing a successful artificial inoculation protocol, collecting seeds from a large number of trees encompassing the range of *A. koa*, establishing seed zones, screening the collected seedlots for genetic resistance, establishing field trials to validate the artificial inoculation results and establishing seed orchards to produce resistant seed for land managers (Sniezko 2003).

Identifying and developing *A. koa* populations that are genetically resistant to virulent strains of *FOXY* may be the key to successful koa restoration and reforestation (Sniezko 2006). In collaboration with the USDA Forest

Figure 1: Healthy *Acacia koa* tree surrounded by trees with koa wilt symptoms in natural stand on Oahu, Hawaii

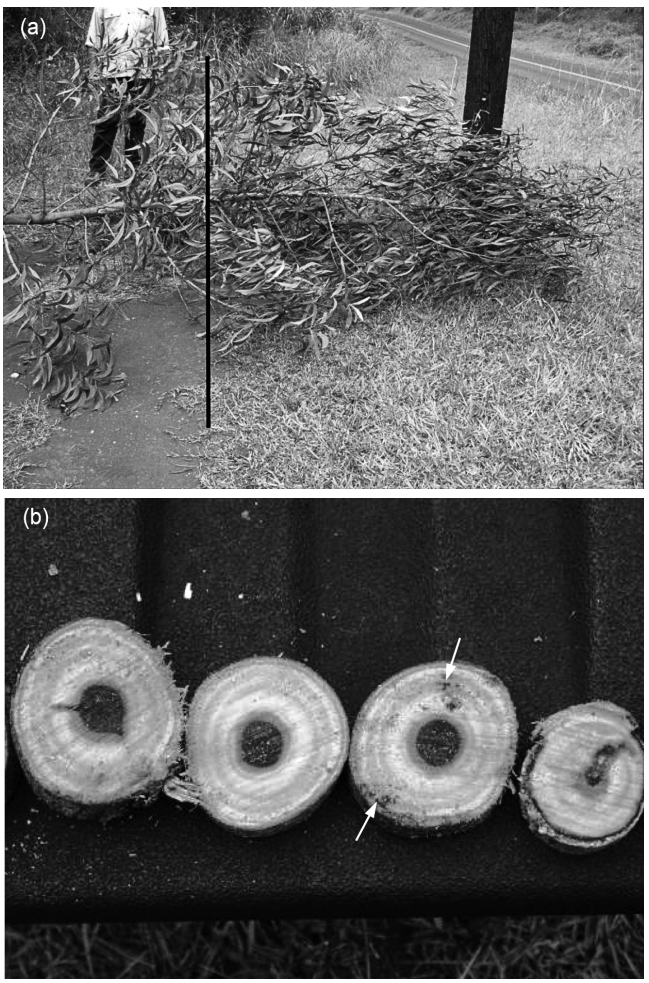

Figure 2: *Acacia koa* on Kauai Island with symptoms of koa wilt disease. (a) Felled *A. koa* tree with dead crown (right of line). (b) Stem dissection of felled tree with vascular discoloration (arrows) at boundary between live and dead section

Service, USDA Natural Resource Conservation Service and the State of Hawai'i, Department of Land and Natural Resources, HARC operates a tree improvement program to develop *A. koa* wilt-resistant populations for multiple ecological zones in Hawai'i. Due to the demand for disease-resistant *A. koa* seed for native forest restoration, HARC is working to develop genetically diverse, wilt-resistant populations for individual ecological regions within the state. The population genetics of *A. koa* are poorly understood and breeding zones are not established. A conservative approach, based on planting locally sourced germplasm, is often a requirement by many restoration programs in the state. Nevertheless, the existing *A. koa* genetic resource in some regions is severely limited and an introduction of germplasm may be necessary for long-term success. Seed zones are not formally established for *A. koa* and seed is primarily collected opportunistically, with little consideration to characteristics of the mother tree.

Survey and isolate collection

A preliminary state-wide survey was conducted to determine the distribution of koa wilt disease within commercial nurseries and field sites on the four largest Hawaiian islands: Kauai, Maui, Oahu and Hawai'i (James et al. 2007a, 2007b). Each sample location was georeferenced and digital maps were developed. Roots and stem/branch sections were dissected into pieces, surface sterilised and placed on selective agar medium (Komada 1975; James et al. 2007a, 2007b). Selected emerging fungi were transferred to potato dextrose and carnation leaf agar for identification using standard taxonomic guides. Percentages of sampled pieces colonised by particular *Fusarium* species were calculated.

Fusarium oxysporum was found to be widely distributed throughout the sampled Hawaiian islands, as it was present at nearly all sampled field and nursery sites (James et al. 2007a, 2007b). While several *Fusarium* species were isolated from roots of trees displaying wilt/dieback symptoms and from nursery seedlings, *FOXY* was by far the most common (Tables 1 and 2). This species was obtained from nearly half of the fine roots sampled, but was less common within larger secondary and tertiary roots. The second-most commonly isolated *Fusarium* species from roots was *F. solani*, which was found on about 10% of sampled roots.

Pathogenicity trials

Since 2005, 165 isolates of *FOXY* collected during the survey were selected for testing in greenhouse seedling inoculation experiments (Dudley et al. 2007, 2013). The primary goal of the pathogenicity trials was to identify highly virulent isolates of *FOXY* for inclusion in disease resistance screening trials. The inoculum preparation methods were adapted from the procedures of Miles and Wilcoxin (1984) and the inoculation process described by Dudley et al. (2007, 2009) was utilised. In summary, very young *A. koa* seedlings were inoculated with individual isolates of *FOXY* in greenhouse trials at HARC's Maunawili Experiment Station. Seedlings were monitored daily and, when seedlings were considered dead (extensive wilting), they were carefully extracted, their roots washed thoroughly to remove adhering particles of growing media, and analysed in the laboratory for root colonisation by inoculated isolates. Re-isolation showed *FOXY* to have colonised the roots of the overwhelming majority (>99%) of the dead seedlings. *FOXY* was isolated from the vast majority of surviving seedlings, indicating isolates were able to enter the root system, regardless of virulence.

The mortality rates varied widely by isolate and served as the basis for assigning virulence. The majority of isolates

Table 1: *Fusarium* spp. root colonisation of diseased *Acacia koa* trees sampled from native forests

	Fine roots	Secondary roots	Tertiary roots	All roots
No. trees sampled	46	33	18	18
No. pieces sampled	763	600	430	1 793
Percentage colonisation				
Fusarium oxysporum	44.4	29.7	23.7	34.5
Fusarium solani	16.2	8.3	6.5	11.3
Other *Fusarium* spp.	9.7	2.3	5.8	6.3
All *Fusarium* spp.	66.6	38.0	34.9	49.4

Table 2: *Fusarium* spp. colonisation of *Acacia koa* seedlings sampled from Hawaiian tree nurseries

	Roots	Stems	All samples
No. seedlings sampled	158	62	162
No. pieces sampled	1 892	533	2 425
Percentage colonisation			
Fusarium oxysporum	56.1	21.2	48.4
Fusarium solani	9.6	8.1	9.2
Fusarium semitectum	7.9	6.0	7.5
Fusarium subglutinans	4.3	4.1	4.3
Fusarium equiseti	1.4	3.6	1.9
Fusarium avenaceum	0.1	3.6	0.8
Other species	2.8	2.6	2.8
All *Fusarium* spp.	77.1	46.1	70.3

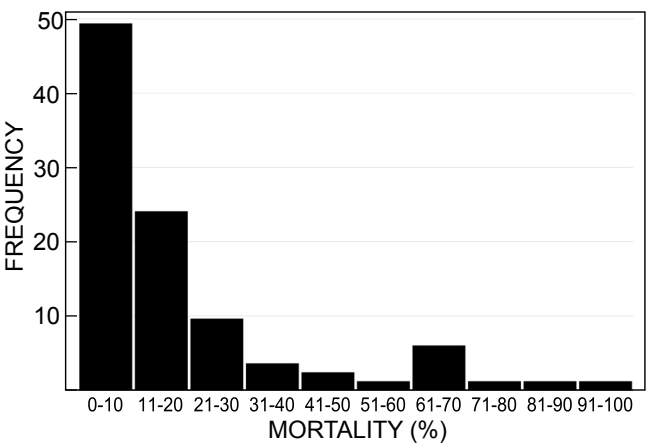

Figure 3: Distribution of mortality of *Acacia koa* seedlings inoculated with 165 isolates of *Fusarium oxysporum* f. sp. *koae* in pathogenicity trials

were non-pathogenic or had low virulence as 79 (48%) of the 165 isolates caused less than 10% mortality (Figure 3). Eleven isolates caused greater than 40% mortality and were classified as highly virulent. At least one isolate from each of the islands sampled caused more than 40% mortality, supporting reports that koa wilt disease is widespread in Hawai'i (Table 3). The mortalities for isolates tested in multiple trials were significantly correlated (Figure 4; $r = 0.90$; $n = 14$; $p < 0.0001$).

Resistance screening

Acacia koa seedpods were collected from individual trees, assigned a unique family name, georeferenced using a handheld GPS and stored at the HARC Maunawili Experiment Station. Mother trees were selected primarily on the basis of seed availability and overall tree health. Trees displaying symptoms of canopy dieback or chlorosis were avoided. Most of the seed was collected from areas of Hawai'i with disease symptoms, but the level of mortality was not recorded for the individual collection areas. While most of the seeds were collected from wild populations, collections were also made from survivors of progeny trials at the HARC Maunawili Experiment Station where high levels of mortality from koa wilt had been documented. Seeds were removed from pods and visually inspected for insect damage prior to storage.

Approximately 100 seeds from each family, including a resistant and susceptible control, were sown and inoculum of the 10 highly virulent isolates was prepared as described for the isolate pathogenicity trials. The ground isolates were thoroughly mixed to yield an inoculum comprised of virulent isolates from a diverse geographic background (Table 3). The inoculum was mixed with growing media and young *A. koa* germinates were transplanted into the inoculated media as described for the pathogenicity trials (Dudley et al. 2009, 2013). The number of seedlings screened from each family varied between the trials, ranging from 25 to 32.

Seedlings were monitored for the development of wilt symptoms 2–3 times per week (Figure 5). Once seedlings were considered dead, the date of mortality was recorded and seedlings were carefully extracted and analysed in the laboratory for root colonisation by *FOXY*, as described for the pathogenicity trials. A random subset of 3–5 seedlings that survived the trial was also prepared for *FOXY* re-isolation. Trials were run for approximately 100 d, based on seedling mortality rates.

As in the prior pathogenicity trials, the seedlings began to show disease symptoms approximately 20 d after transplant into inoculated media. The mortality peaked between 30 and 60 d after transplanting. By day 100, the mortality slowed dramatically and the trials were terminated. The overall survival was approximately 45%, but varied greatly by family (Figure 6). Rates of survival for the top families were over 75%, whereas the most susceptible families had zero percent survival. The trials have proven effective in distinguishing resistance frequency by family in a relatively

Table 3: Virulent *Fusarium oxysporum* f. sp. *koae* isolates identified in pathogenicity trials. All isolates were collected from symptomatic *Acacia koa*

Isolate	Collection location	Host information	Mortality (%)[a]
0503J	Hawaii Island	Fine tree roots; wilted	72, 60
0505A	Hawaii Island	Fine tree roots; wilted	44, 40
0562B	Kauai	Fine tree roots; wilted	60, 44
0563O	Kauai	Rhizosphere; wilted tree	80, 52
0562D	Kauai	Stem; outer; wilted tree	88, 92
0562E	Kauai	Stem; inner; wilted tree	68, 72
0562G	Kauai[b]	Stem; outer; wilted tree	64
0602J	Oahu	Seeds/young germinants	44
0602K	Oahu	Seeds/young germinants	68, 56
0602L	Oahu	Seeds/young germinants	56
0545B	Maui	Roots; wilted tree	60

[a] Isolates with two mortality values were tested in two separate pathogenicity trials

[b] Isolate died in culture, not used in subsequent resistance screening trials

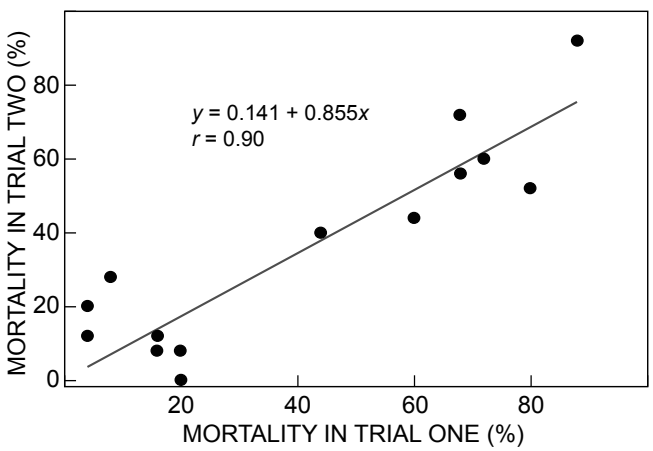

Figure 4: Comparison of percentage mortality in two pathogenicity trials of *Acacia koa* seedlings inoculated with isolates of *Fusarium oxysporum* f. sp. *koae*

Figure 5: Healthy and wilted *Acacia koa* seedlings inoculated with virulent isolates of *Fusarium oxysporum* f. sp. *koae*

short time frame. In addition, field validation of screening results is essential and is underway.

Since 2011, three primary koa populations have been screened for wilt resistance: Koolau Mountain Range on Oahu, Southeast Mauna Loa on Hawai'i Island and windward Haleakala on Maui. These populations were selected because there is a demand for wilt-resistant seed for these areas of the state, and many landowners/managers have a strong preference for planting seed from local populations. Approximately 100 families from the Hawai'i and Maui islands populations were initially included in the two screening trials, but poor germination and high rates of seed predation reduced the number screened to approximately 80 families for each population. For the Oahu Koolau population, approximately 150 families were screened in three trials. In total approximately 310 families were screened for these three regions. The results of the screening trials were then used to select families for wilt-resistant field trials in those regions (Figure 7). A previously identified susceptible family and resistant family were included in all trials as a control. The susceptible control family was collected from Kauai Island and the resistant control was collected from Oahu.

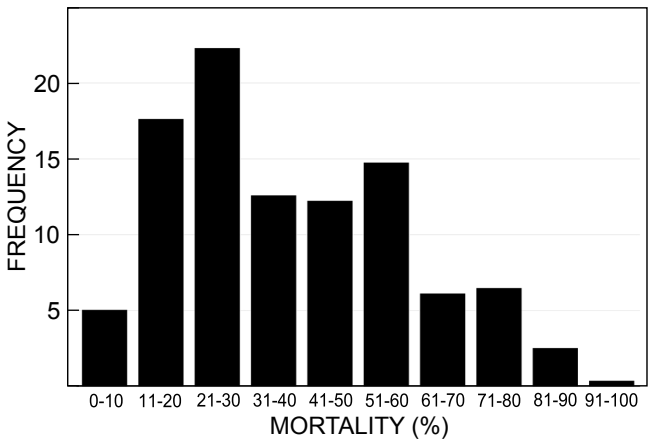

Figure 6: Overall family survival distribution of *Acacia koa* families screened in *Fusarium oxysporum* f. sp. *koae* seedling screening trials

Vegetative propagation of wilt-resistant seedlings

Vegetative propagation of forest tree species has become increasingly important in commercial forestry programs worldwide. Vegetative propagation of selected individuals is a powerful tool with implications beyond clonal forestry. At the heart of the advantages is the ability to capture all the genetic variance of selected individuals and therefore

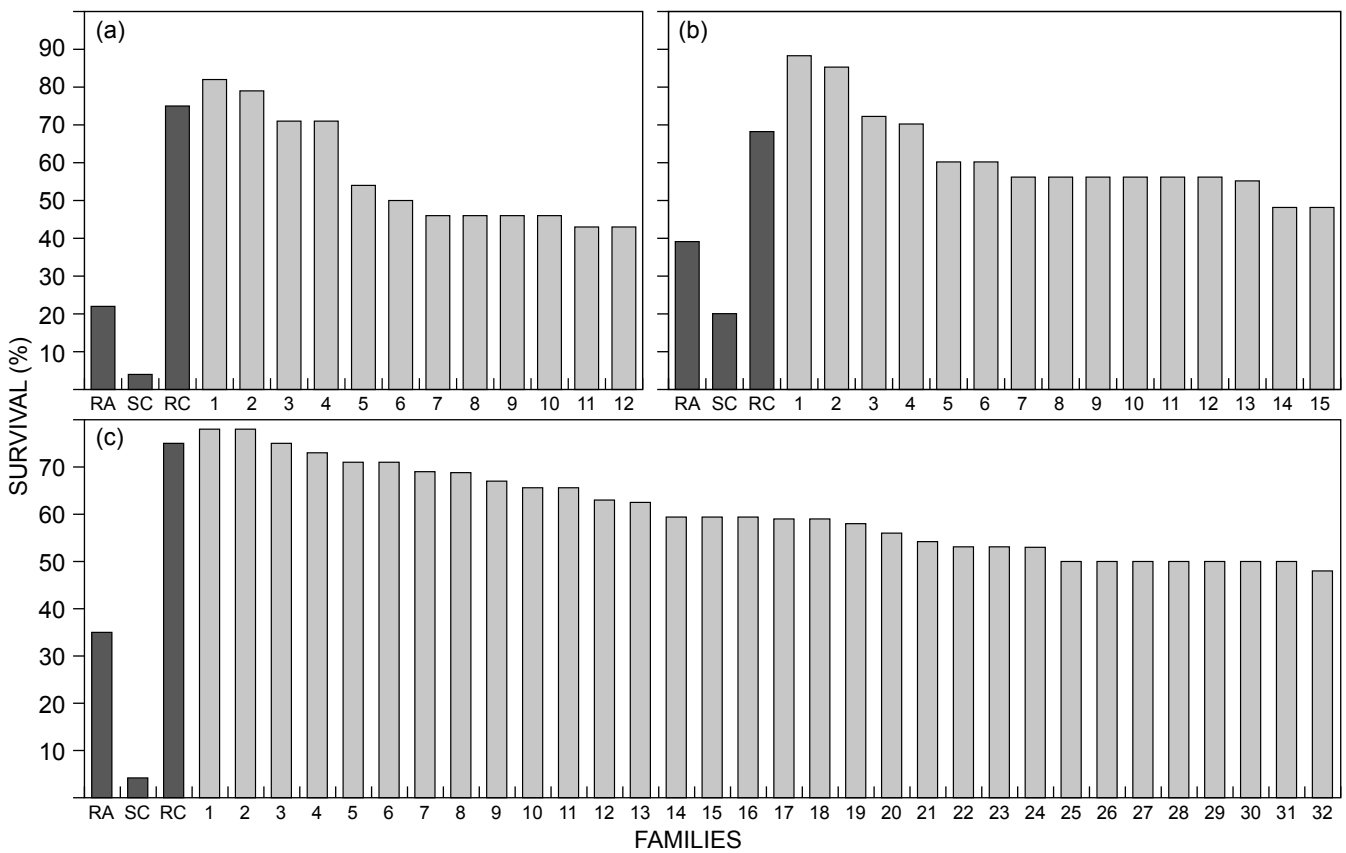

Figure 7: Percentage survival of *Acacia koa* seedlings selected for field trials from *Fusarium oxysporum* f. sp. *koae* resistance screening trials. Populations of *A. koa* from three regions of Hawaii were screened: (a) Southeast Mauna Loa, Hawaii Island; (b) Windward Haleakala; and (c) Koolau Mountain Range, Oahu. The same susceptible and resistant control (SC and RC) families were used in each trial. The regional average (RA) is the average survival for all families screened from the region

increase genetic gain. The primary uses for vegetative propagation include large-scale deployment of elite clones, genetic testing, clonal seed orchards, clonal family forestry and preservation of unique genotypes (Libby and Ahuja 1993).

Previous research on *A. koa* and other tree species suggests that successful root formation in stem cuttings is under genetic control (Skolmen 1977; Shi 2003). Skolmen and Shi investigated the impact of several plant hormones at a range of concentrations on rooting success, but no optimal treatment was determined. The genetic variation in rooting potential suggests that high rooting genotypes must be selected before rooted cuttings are a viable option for clonal production. The specific objective of this project was to develop a method for selecting high rooting, wilt-resistant genotypes. Once selected, stock plants of these genotypes would serve as the source for propagating large numbers of wilt-resistant clones.

Survivors from the wilt screening trials were transplanted into larger containers and grown in full sun on nursery benches at HARC's Maunawili Experiment Station. The seedlings were monitored for insects and pathogens and infected seedlings were removed. The primary pest was the black twig borer (*Xylosandrus compactus*), which caused greater losses during late summer months. Four to ten, two-node stem cuttings were taken from each seedling. The cuttings were examined after five weeks and the percentage of cuttings that successfully rooted was determined. Rooted cuttings were transplanted into growing containers and then placed on greenhouse benches. After one month, the cuttings were moved outside onto nursery benches under full sun.

Three-hundred surviving seedlings from the Koolau Mountain Range, Oahu wilt screening trial were transplanted in June 2011. At least one healthy, vigorous seedling was selected from 59 families for testing. Cuttings were taken from August to November 2011. Of the 300 seedlings, cuttings from 190 (63%) did not root successfully, and while cuttings from another 40 (13%) rooted, the success rate was 25% or lower (Table 4). Therefore, 230 seedlings were discarded due to no/low rooting. Cuttings from 70 (23%) seedlings all had greater than 25% rooting success and were selected for further propagation. Additional stem cuttings were rooted from the best rooting genotypes to provide plant material for a clonal field trial.

Two-hundred and seventy-five surviving seedlings from a Hawai'i Island wilt screening trial and 165 seedlings from a Maui Island screening trial were tested for rooting ability. Rooting success was lower than that of the Oahu population and it was not feasible to produce sufficient plant material for clonal trials for these populations (Table 4). It is unclear if the decreased rooting ability was caused by a lower genetic potential for rooting or differing environmental factors as the rooting environment was not climate controlled.

Table 4: Summary of rooting ability of wilt-resistant *Acacia koa* for three populations

Population	No. of stock plants screened	Rooting success of stock plants (no. of plants; %)		
		0% Rooting	1–25% Rooting	Over 25% Rooting
Oahu	300	190 (63%)	40 (13%)	70 (23%)
Hawaii Island	275	184 (67%)	62 (23%)	29 (11%)
Maui	165	128 (78%)	11 (7%)	26 (16%)

Wilt-resistant *Acacia koa* field trials

Field sites were selected for planting wilt-resistant families identified in greenhouse inoculation trials. An additional site was selected for testing wilt-resistant clones produced from rooting stem cuttings. In 2012–2014, HARC planted two trials on Hawai'i Island, one on Maui and two on Oahu (Table 5). Each field trial was planted with families or clones originating from trees in the surrounding region. Yearly survival and growth data were collected for each site and will continue into the future. The early survival data indicates that there is a significant improvement over previous trials using unscreened families, particularly at the Oahu site, where disease pressure is known to be high. Several of the seedlings from the susceptible control family died in 2013 at the Oahu site, but continued monitoring and data collection is critical to understand the durability of resistance. Several of the trials will be thinned in late 2014 and 2015. If survival remains high, thinning will be based primarily on growth characteristics such as stem form and volume (Figure 8).

Seed production

The putative wilt-resistant plantings on Hawai'i Island, Maui and Oahu are managed by HARC and project cooperators. The plantings will be thinned over the next several years based on survival and growth performance. The trees should begin to produce seed 3–5 years after planting, with production increasing as the trees age. HARC has established relationships with the landowners to coordinate seed collection and processing. As seed becomes available, HARC anticipates releasing the seed to the

Table 5: Summary of *Acacia koa* wilt-resistant field trials

Site location	Elevation (m)	Germplasm	Planting design	Planting date	Survival at 12 months (%)
Kapapala, Hawai'i Island	1 100	12 families[a]	Replicated row plots	May 2012	99
Glenwood, Hawai'i Island	750	12 families[a]	Replicated row plots	February 2013	96
Leeward Haleakala, Maui	1 300	15 families	Replicated row plots	September 2013	n/a
Maunawili, Oahu	150	34 families	Replicated row plots	September 2012	70[b]
Maunawili, Oahu	150	26 clones	Replicated single tree plots	January 2013	97

[a] Same families at both sites

[b] Majority of mortality within one month of planting and not attributed to koa wilt disease

public. The ability to provide genetically appropriate seed for future *A. koa* reforestation and restoration efforts will be a significant improvement over the current practice of utilising unimproved seed collected opportunistically.

Discussion

The limitations of field trials were the impetus for developing the greenhouse inoculation methods for wilt screening. The screening trials were able to identify differences in survival between *A. koa* families in response to inoculation with virulent isolates of *FOXY*. The survival rates provide the basis for selecting resistant families to serve as a base population for *A. koa* breeding programs. Continued monitoring of the field plantings is critical in order to determine the long-term survival in the field and ensure its correlation to the resistance selected in young seedlings. The resistant and susceptible controls were consistently among the best and worst families in each trial, indicating consistency between trials. The re-isolation of the pathogen from within the surviving seedlings provides some insight into the host–pathogen interaction, indicating the pathogen was able to infect the plant but not cause mortality. A better understanding of the plant–host interaction and the mode of inheritance for resistance gene(s) would help to guide future breeding efforts.

The ability to rapidly screen a large number of *A. koa* families at relatively low costs makes the methods presented a powerful tool for tree improvement. While a relatively small number of improved families or clones may be sufficient to meet the needs of commercial forestry, *A. koa* restoration efforts should be based on disease-resistant families that approximate the range of genetic diversity found in natural populations. To accomplish this goal, it is necessary to identify a large number of wilt-resistant families. Therefore, a rapid screening method is the only practical option. The goal of establishing wilt-resistant seed orchards comprised of locally sourced germplasm in numerous ecological regions throughout the state is now underway. HARC will concentrate on establishing new wilt-resistant populations in new regions of the state. The result of this endeavour is locally adapted, ecoregion-specific seed that allows for the restoration of this iconic species and commercial reforestation opportunities.

While most of the previous research has focused on developing wilt-resistant *A. koa* for areas where the disease pressure is strong (low- to mid-elevation sites), identifying wilt-resistant, higher-elevation populations should not be

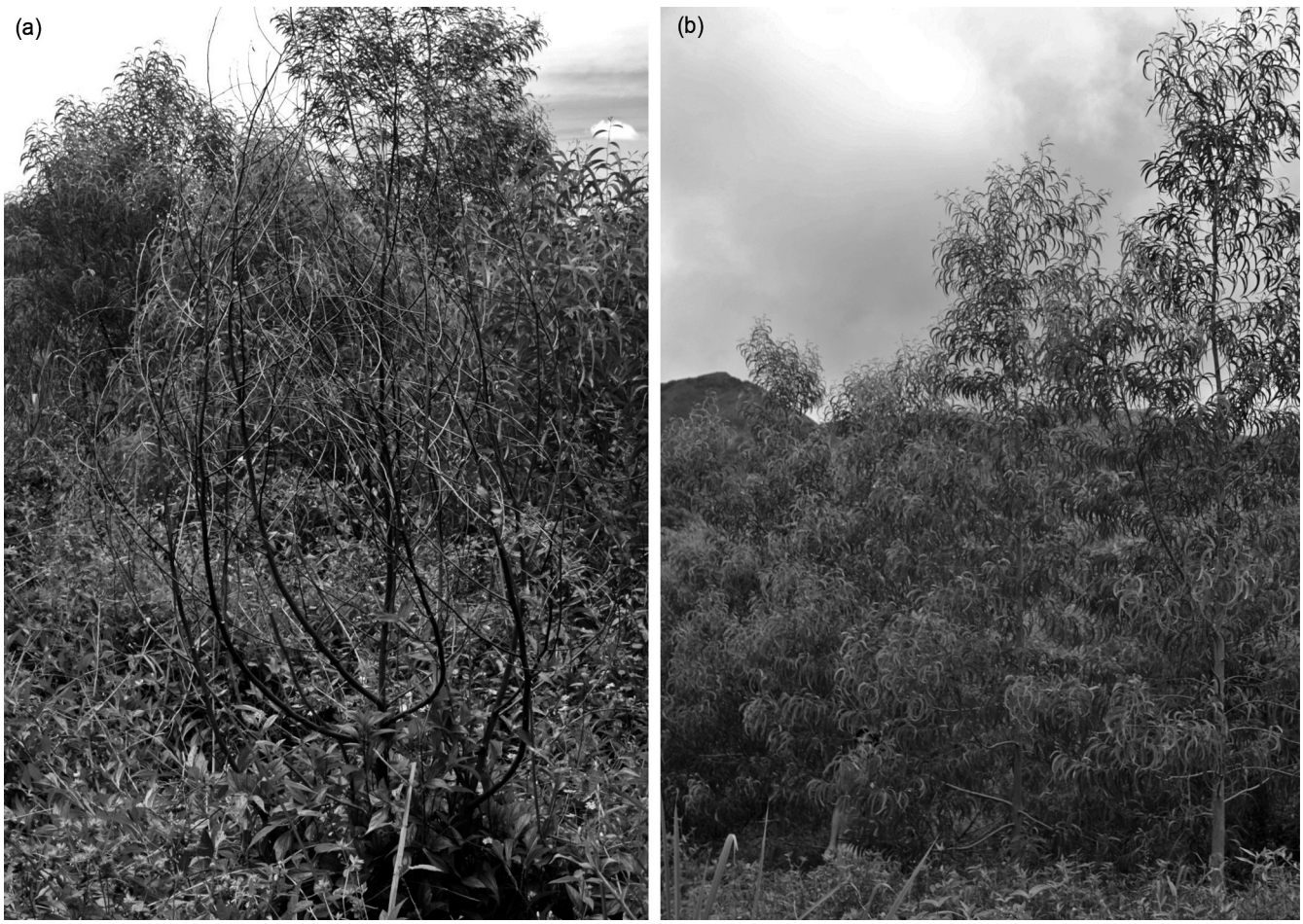

Figure 8: *Acacia koa* field planting on Oahu, Hawaii at 18 months after planting. (a) Dead susceptible control family; (b) wilt-resistant *A. koa* with superior stem form

overlooked. Recent surveys by James et al. (2007a) found *FOXY* to be widely distributed across the state, and Stoner et al. (1975) found *FOXY* in koa forests as high as 1 800 m. The higher-elevation populations have not shown increased resistance in greenhouse inoculation trials. The effect of soil temperature on the incidence of *FOXY* has been well studied in numerous plant hosts. Disease severity is typically suppressed at lower temperatures and is typically greatest at 25–30 °C (Clayton 1923; Scott et al. 2001; Landa et al. 2006). The effect of soil temperature on *FOXY* virulence in *A. koa* has not been studied, but the cooler temperature at higher elevations is a logical explanation for the decreased severity of koa wilt disease. Global climate change and the subsequent increase in soil temperature threatens to increase the disease severity in areas where it is currently limited. Developing wilt-resistant, high-elevation seed sources will provide a level of biosecurity in case of such an event and ensure that *A. koa* remains a keystone species in remaining Hawaiian native forests.

Finally, the screening method has great potential for developing *A. koa* with improved commercial traits for plantation forestry. Previous efforts to select families with improved traits were greatly hindered because the majority of trees were killed by koa wilt disease. The ability to screen for disease resistance prior to planting genetic field trials will ensure higher survival and make selection of other traits such as growth rate, tree form and wood quality possible.

The methods presented enabled vegetative propagation of wilt-resistant clones for genetic testing, but the low rooting percentage is suboptimal for operational forestry. There is strong potential that rooting percentage could be increased significantly through the development of improved propagation techniques. Through applied research, rooting percentages exceeding 90% have been achieved in other *Acacia* species (Kha 2001). The ability to vegetatively propagate disease-resistant genotypes allows for the establishment of clonal field trials in order to select elite clones that exhibit several traits of economic importance. Clones may prove particularly useful in *A. koa* improvement due to the high level of heterozygosity, complex mode of inheritance and the relatively large number of traits that need improvement. Vegetative propagation is also a valuable tool for conducting research trials to understand the genetic control of particular traits and genotype by environment effects.

Conclusions

Substantial progress has been made in identifying *FOXY*-resistant *A. koa* families through greenhouse screening trials. This is a critical step to meet the overall objectives of protecting the remnant *A. koa* forests, restoring its former native range, ensuring a sustainable supply of timber, and significantly reducing risk and uncertainly of reforestation and restoration efforts. Field trials of resistant families have been established and early results suggest substantial improvement in survival in the field relative to unscreened material. Long-term monitoring of field plantings is critical to determine the durability of wilt resistance. Strategies to mass-propagate resistant varieties for restoration and commercial plantations via seed and vegetative propagation methods are under development.

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