

# MOLECULAR GENETIC CHARACTERIZATION OF THE KOA-WILT PATHOGEN (*FUSARIUM OXYSPORUM*): APPLICATION OF MOLECULAR GENETIC TOOLS TOWARD IMPROVING KOA RESTORATION IN HAWAI'I

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## INTRODUCTION

Several forest diseases are causing serious threats to the native Hawaiian forest. Among them, koa-wilt disease (caused by *Fusarium oxysporum*) is damaging to native populations of koa (*Acacia koa*), and it also hinders koa restoration/reforestation. Because *F. oxysporum* likely represents a complex of species with distinct pathogenic activities, more detailed characterization is needed to better understand the *F. oxysporum* that is associated with koa wilt. Such data would allow assessments of genetic diversity and population structure, while also providing inferences as to whether the pathogen is indigenous and/or potential modes of spread. DNA-based characterization of the koa-wilt pathogen will allow assessments of pathogen populations to inform koa restoration efforts, and assist resistance-breeding and screening programs for koa.

## Importance of Koa (*Acacia Koa*) In Hawai'i

### (A) Ecological importance

- Provides habitat for many native bird and insect species.

- Predominate nitrogen-fixing, native tree species.
- Critical role in watershed health and rehabilitation.
- Dominant tree species in many native ecosystems.

### (B) Cultural importance

- Native Hawaiian's primary wood for canoes.
- Tannins from bark used as red dye for kapa cloth.
- Integral part of many Hawaiian ceremonies.

### (C) Economic importance

- Koa represents 90% of the Hawaiian Forest Products Industry.
- Valued at \$30 million annually (2001).
- Harvested primarily from native forests.
- Koa is the preferred wood of the Hawaiian Specialty Hardwood Market.
- Koa is one of the most valuable woods in the world, with prices exceeding \$125 per board foot.

## The Problem – Koa Wilt Disease

- Koa wilt disease causes high rates of mortality in koa plantings.
- Capable of killing trees of all ages in native forests.
- Causal agent of koa wilt disease - *Fusarium oxysporum* f. species *koae*.
  - ✓ First described in koa by Gardner in 1980.
  - ✓ Species infects wide range of plants, but non-pathogenic and pathogenic isolates cannot be distinguished morphologically.
  - ✓ Little information is available on genetic characterization of koa wilt pathogen.

In: Ramsey, A. & P. Palacios (Comps). Proceedings of the 63rd Annual Western International Forest Disease Work Conference; 2015 Sept. 21-15; Newport, OR. <sup>1</sup>Kookmin University, Department of Forestry, Environment and Systems, Seoul, South Korea. <sup>2</sup>Colorado State University, Department of Bioagricultural Sciences and Pest Management, Fort Collins, Colorado <sup>3</sup>Hawaii Agriculture Research Center, Maunawili Research Station, Kailua, HI <sup>4</sup>USDA Forest Service, Forest Health Protection, Region 5, Vallejo, CALIFORNIA <sup>5</sup>Plant Disease Consulting, Vancouver, Washington <sup>6</sup>USDA Forest Service, Rocky Mountain Research Station, Moscow, Idaho.

A)



B)



**Figure 1 (A, B)** Koa wilt disease caused by *Fusarium oxysporum*.

### Molecular Methods to Characterize Koa Wilt Pathogen

The Hawaii Agriculture Research Center collected many isolates of koa wilt pathogen and we will visit more sites to collect additional samples for

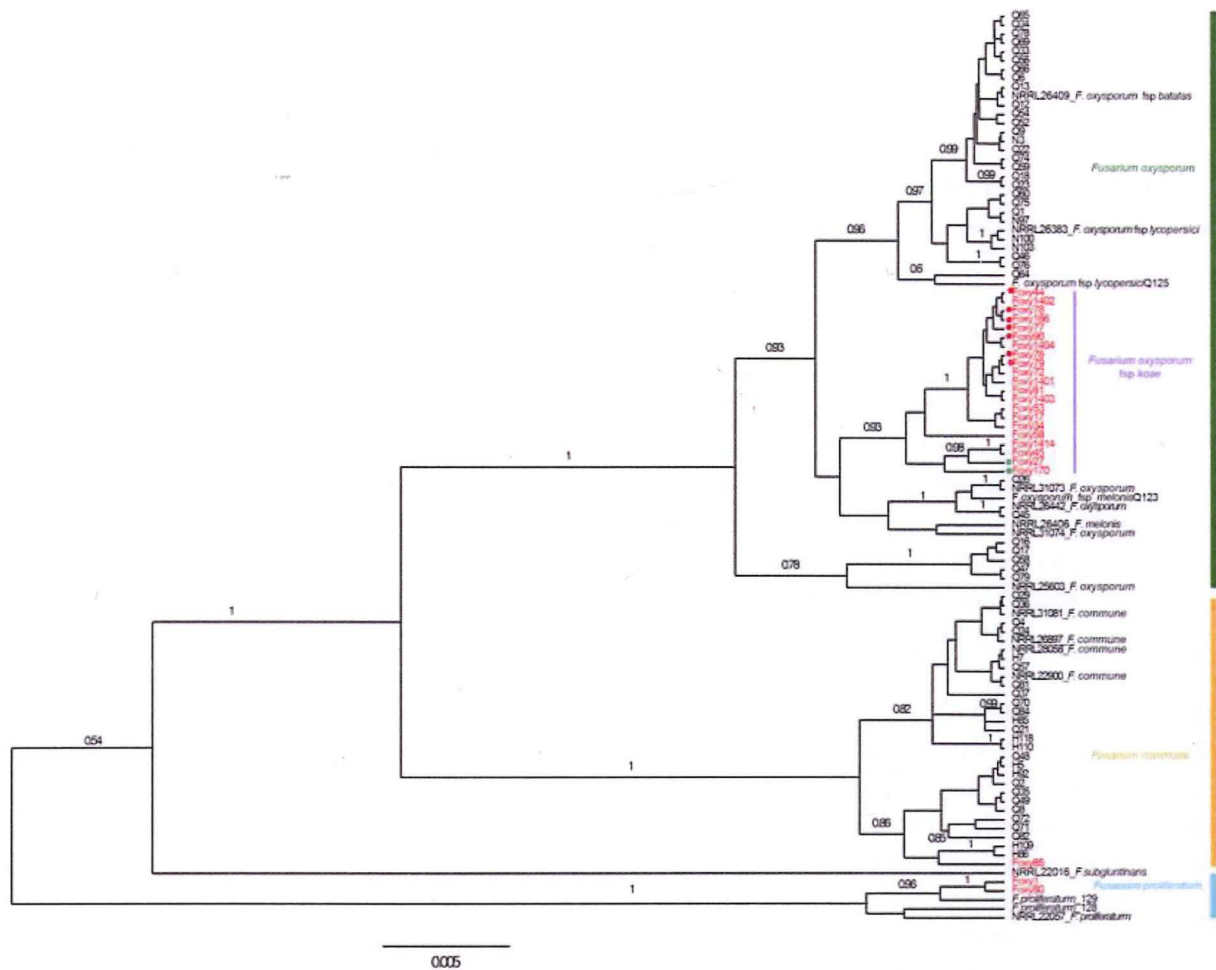
genetic characterization. Because *F. oxysporum* likely represents a complex of species with distinct pathogenic activities, more detailed characterization is needed to better understand the *F. oxysporum* "subspecies" (phylogenetic clade) that is associated with koa wilt. Initial characterization will be accomplished with phylogenetic analysis of multiple gene regions and Restriction Associated DNA sequencing (RAD-seq), which will be used to further characterize the koa wilt pathogen populations in Hawai'i. Such data would allow assessments of genetic diversity and population structure, while also providing inferences as to whether the pathogen is indigenous and/or potential modes of spread.

### PRELIMINARY RESULTS

- A total of 24 *Fusarium* spp isolates (indicated in red; previously identified as *F. oxysporum* based on morphology) collected from diverse substrate associated with diseased and healthy koa in Hawai'i. All the isolates were sequenced for the translation elongation factor 1 $\alpha$  (*tef-1 $\alpha$* ) gene.

- All the isolates clustered together except three isolates (Foxy85 – *F. commune* and Foxy1 and Foxy80 – *F. proliferatum*). Some *Fusarium* isolates went through pathogenicity tests (red dots - highly virulent isolates vs. green dots non-pathogenic isolates) (Figure 2).

- Three *Fusarium* spp identified by *tef-1 $\alpha$*  were associated with koa wilt. We plan to 1) collect more *Fusarium* spp isolates associated with koa wilt across Hawai'i; 2) identify isolate at the species and/or subspecies level; 3) conduct pathogenicity tests of selected isolates from each species/subspecies; 4) develop diagnostic primers for detecting pathogenic *F. oxysporum* f. species *koae*; 5) assess genetic diversity and population structure of *F. oxysporum* f. species *koae* using RAD-seq; and 5) provide a guidance to koa wilt breeding resistance program based on results from this project.



**Figure 2.** Consensus phylogeny of coalescence-based Bayesian analyses estimated in BEAST under the strict clock with a GTR model of substitution on the translation elongation factor 1 $\alpha$  gene consensus (50% strict) sequences of 24 *Fusarium* spp isolates (colored in red). Posterior support values >0.5 are indicated at the nodes.

## REFERENCE

Gardner, D.E. 1980. *Acacia koa* seedling wilt caused by *Fusarium oxysporum* f. species *koa*, f. species nov. Phytopathology 70:594-597.