

# DEVELOPMENT OF TOOLS FOR EARLY DETECTION, MONITORING AND MANAGEMENT OF THE KOA WILT PATHOGEN (*FUSARIUM OXYSPORUM* F. SP. *KOAE*) IN HAWAI'I

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## INTRODUCTION

Koa (*Acacia koa* Gray) is an endemic, keystone species in Hawai'i's forests. Koa is valuable economically (contributed \$30 million to Hawai'i's forestry industry in 2001), ecologically (habitat for many endangered birds and insects), and culturally (koa is the main wood used for making Hawaiian canoes). Mortality of koa trees due to koa wilt (caused by *Fusarium oxysporum* f. sp. *koae*; Foxy-koae) has been increasing, primarily in the low- to mid-elevation forests (Gardner 1980). *Fusarium oxysporum* (Foxy) is an important vascular wilt pathogen of many plant species worldwide (Leslie 2006). Foxy is highly variable and can be pathogenic or saprophytic without discernable, morphological differences. The origin of Foxy-koae strains that are virulent to koa in Hawaii is currently unknown.

Research conducted by Stewart et al. (2006, 2012), showed that highly virulent strains of Foxy could be identified by DNA sequences. The resulting DNA marker enhances the ability to identify the pathogen in nursery systems (Stewart et al 2006, Stewart et al 2012), and methods and markers to detect and identify pathogenic *Fusarium spp.* have been tested in tree nursery systems (Leon 2015). Molecular characterization of Foxy-koae will contribute a better understanding of its biology and ecology for use in further restoration and management efforts.

Development of DNA-based probes that differentiate pathogenic from non-pathogenic Foxy isolates will allow for quick, reliable detection of pathogenic strains of Foxy-koae. A methodology to rapidly detect and quantify pathogenic Foxy in nursery substrates, wood tissue, seedlings, and forest soils using real-time PCR will allow in the implementation of this management technique. Furthermore, an extensive record of newly collected isolates from forest sites will help determine conditions conducive to disease development. This information will allow the development of a recommended protocol to enable foresters and nursery managers to implement timely and appropriate disease management practices.

## METHODS

Pathogenicity information of existing Foxy-koae isolates are being used to determine if genetic differentiation exists between pathogenic and non-pathogenic strains. If a clear distinction is found, new isolates will be separated into these two groups. Molecular real-time PCR probes will be developed to identify and quantify pathogenic Foxy-koae isolates. New isolates collected from root and soil samples from symptomatic koa trees

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(exhibiting chlorotic leaves, crown dieback, and dark staining of infected roots – Figure 1) and asymptomatic koa trees will be screened with the molecular probes to verify the presence of pathogenic or non-pathogenic strains of Foxy-koae. To confirm the pathogenicity groups, 20 isolates from each group will be selected for pathogenicity trials, using granulated Foxy-koae inoculum under greenhouse conditions. Protocols and guidelines for koa breeding and restoration programs will be developed based on the estimated population levels of pathogenic Foxy-koae isolates.



To determine virulence variation and genetic diversity, at least 100 new *Fusarium* isolates will be collected from Hawai'i. Isolates will be collected across the main Hawaiian Islands and from various locations within each island to determine variability among and within islands. Initial characterization will be accomplished with phylogenetic analysis of multiple gene regions and Restriction-Site-Associated-DNA sequencing (RADseq). Environmental conditions (such as GPS coordinates, site conditions, slope, climate data, etc) will be collected at each site for examinations of environmental relationships with koa wilt.

**Figure 1.** 8-year-old Acacia koa tree showing wilt symptoms (crown dieback, yellowing leaves) caused by *Fusarium oxysporum* f sp. *Koa*.

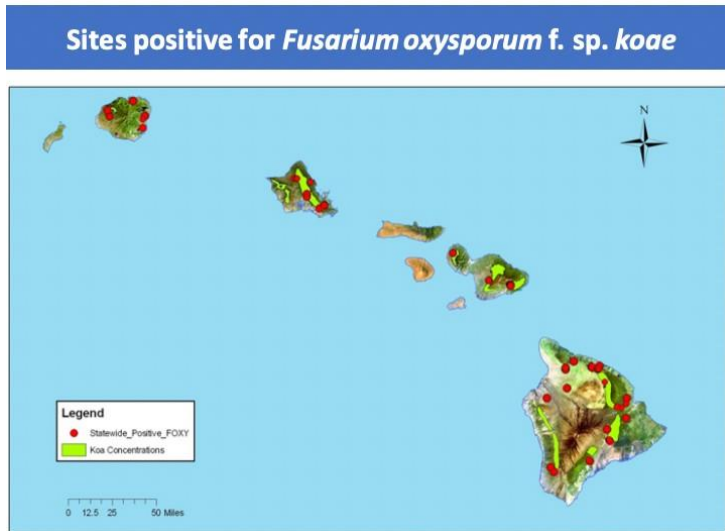
## RESULTS

The Hawai'i Agriculture Research Center (HARC) has conducted surveys around the main Hawaiian Islands and found koa wilt across the islands (Figure 2). Over 200 isolates of Foxy-koae have been recovered from symptomatic trees in many of the native forests and restoration sites that were surveyed on the islands of Oahu, Maui, Kauai and Hawai'i. Some of these isolates have been screened for pathogenicity.

Twenty-one screened Foxy-koae isolates were genetically analyzed at the USDA Forest Service Forest Pathology laboratory in Moscow, Idaho in 2015 (see Figure 3). The translation elongation factor-1a (*tefl*) locus and mitochondrial small subunit (mtSSU) (Stewart et al. 2006) have been used to identify single nucleotide polymorphisms (SNPs). Two SNPs from the *tefl* and three SNPs from the mtSSU have been identified at these regions.

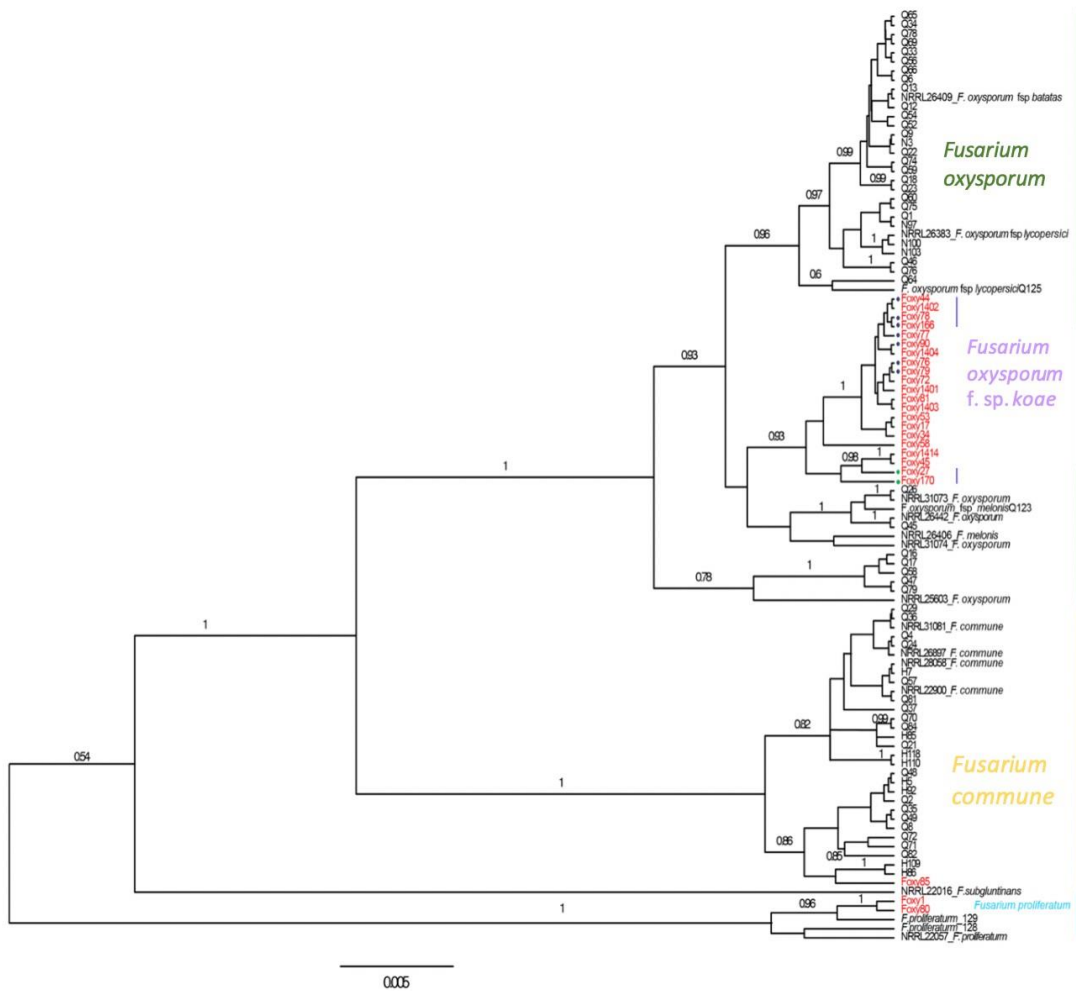
## DISCUSSION

SNPs analyses have revealed a distinct clade of Foxy-koae isolates, which further confirms that these pathogenic isolates are genetically more similar to each other than other *Fusarium oxysporum* isolates. Further characterization of the genome is needed to develop a molecular marker to distinguish pathogenic from non-pathogenic strains because only a few SNPs have been identified in the *tefl* and mtSSU regions. Other regions of the genome will be analyzed to identify genomic areas that are clearly differentiated for marker



development. After the development of a diagnostic marker, newly collected Foxy isolates will be screened for pathogenicity. Isolates found to be virulent (causes koa wilt symptoms – Figures 4 and 5) will be used in disease resistance screening programs. New isolates will be collected in spring or summer of 2018 for use with a molecular marker that is being developed.

**Figure 2.** HARC survey of Hawaiian Islands for *Fusarium oxysporum* f. sp. *koeae* from 2004-2007.



**Figure 3.** Consensus phylogeny of *Fusarium* sequences using coalescence-based Bayesian Analysis estimated in Evolutionary Analysis by Sampling Trees (BEAST). In red – 24 *Fusarium* spp. isolates from *Acacia koa* trees from Hawai'i sequenced at *tef-1a*.



**Figure 4.** Wilted *Acacia koa* seedling caused by *Fusarium oxysporum* f sp. *koae*.



**Figure 5.** Healthy *Acacia koa* seedling.

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