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COLONIZATION OF DISEASED *ACACIA KOA* TREES WITH *FUSARIUM* SPECIES

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ABSTRACT

Fifty-two *Acacia koa* trees with wilt/dieback disease symptoms in Hawaii were sampled for associated *Fusarium* spp. within their roots, stems and branches. *Fusarium oxysporum* was isolated from nearly 35% of all sampled roots and was especially common on healthy-appearing fine feeder roots. *Fusarium solani* was isolated most frequently from stem and branch tissues, but also occurred on more than 10% of sampled roots. A total of fourteen *Fusarium* spp. were detected within diseased trees; we suspect that most of these were probably non-pathogenic fungal endophytes. We found that *F. oxysporum* commonly colonized diseased trees, especially roots. Coupled with our results from pathogenicity testing, we believe that this fungal species is probably important in koa wilt/dieback disease etiology.

INTRODUCTION

Acacia koa A. Gray is one of the most important native forest trees in Hawaii. Koa wood is highly prized for several important products, including surf boards, paneling, furniture, and miscellaneous carvings. This tree species grows very quickly under Hawaiian forest conditions and high-valued trees can be produced in relatively short rotations (Dudley 2002).

An important disease resulting in wilt and dieback symptoms was first described on koa in 1980 (Gardner 1980). Etiological studies determined that the primary cause of the disease was root-infecting strains of *Fusarium oxysporum* Schlecht. (Anderson and others 2002; Gardner 1980). Isolates capable of eliciting disease symptoms were placed within the taxon

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F. oxysporum f.sp. *koae*. Subsequent work evaluated ecological factors important in disease occurrence, spread and intensity (Anderson and others 2002). In addition, one study (Daehler and Dudley 2002) found that the black twig borer [*Xylosandrus compactus* Eichhoff (Coleoptera: Scolytidae)] often infested declining trees and was commonly associated with colonization of stem and branch wood by another *Fusarium* species [*F. solani* (Mart.) Appel & Wollenw.].

Although Koch's postulates have been completed several times, confirming pathogenicity of *F. oxysporum* on koa, controversy still remains regarding the actual importance of this fungus in disease etiology. We have completed several greenhouse pathogenicity tests and found that certain isolates of *F. oxysporum* are highly virulent on susceptible koa seedlots (Dudley and others 2007a), although the proportion of isolates from populations associated with diseased trees that are capable of being aggressive pathogens is relatively low.

Although some reports indicated that *F. oxysporum* may infest koa seeds (Gardner 1980; Anderson and others 2002), we have only detected this species at very low levels on or within seeds (James 2004; James and others 2006). However, several other *Fusarium* spp. have routinely been isolated from koa seeds (James 2004; James and others 2006).

An important part of our investigations concerning koa wilt/dieback disease included disease-impact surveys on the four major Hawaiian Islands (Oahu, Kauai, Maui, and Hawaii). The goal of these surveys was to determine distribution and severity of the disease throughout the natural and planted range of koa. As an important part of these surveys, isolations were made from diseased trees to determine occurrence and extent of colonization by *F. oxysporum* and other *Fusarium* spp. that may influence disease etiology. This report summarizes our findings regarding colonization of diseased koa trees by *Fusarium*.

MATERIALS AND METHODS

Koa trees exhibiting external symptoms of wilt and/or dieback disease were located primarily via roadside surveys or from information provided by landowners. Fifty-two trees were selected based on external indications of disease, including foliar necrosis, dieback, and wilting. Selected trees were also examined for definitive green-black staining within the center of stems, branches and large roots, which we have found commonly associated with the disease. Wood samples from symptomatic trees were collected from selected branches, portions of the main stem, and from root systems. Emphasis was placed on collecting root samples, particularly fine feeder roots, since this is likely the point of infection by wilt-inducing organisms. Collected samples were kept cool within ice chests and subsequently washed thoroughly to remove soil and shipped to the laboratory for analysis.

In the laboratory, samples were divided into five categories (branches, stems, fine roots, secondary roots, and tertiary roots); the latter two categories were selected based on branching pattern and size of roots. All samples were thoroughly rinsed again under running tap water. Small pieces of wood (approximately 5 mm^2) were aseptically extracted from samples, surface sterilized in 0.525% aqueous sodium hypochlorite (10% bleach solution), rinsed in sterile water, and placed on a selective agar medium for *Fusarium* and closely-related fungi (Komada 1975). Fine roots were dissected into pieces about 5 mm long and randomly-selected for placement on the selective medium following surface sterilization. Plates were incubated under diurnal cycles of cool, fluorescent light at about 24°C for 7-14 days. Selected emerging fungi were single-spored and transferred to potato dextrose and carnation leaf agar (Fisher and others 1982) for identification of associated *Fusarium* spp. using the taxonomy of Nelson and others 1981. Percent colonization of wood samples by different *Fusarium* spp. was calculated.

RESULTS

Twelve different *Fusarium* species were isolated from roots of trees displaying wilt/dieback symptoms (Table 1). By far the most common *Fusarium* species isolated from roots was *F. oxysporum*. 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 slightly more than 11% of sampled root pieces. The ten other *Fusarium* spp. were isolated infrequently. Overall, *Fusarium* spp. were obtained from about 2/3 of the fine root pieces and nearly half of all sampled root pieces (Table 1).

Fusarium spp. colonized stem and branch tissues at lower levels than roots (Table 2), although a wide diversity of species were isolated. The most commonly isolated *Fusarium* species was *F. solani*, followed by *F. oxysporum* and *F. proliferatum* (Matsushima) Nirenberg. *Fusarium* spp. were about equally distributed throughout stem and branch tissues, i.e., about the same colonization levels were detected within tissues near the center (usually adjacent to the green-black staining) and tissues just inside the cambium (Table 2). Slightly less than a third of all sampled wood pieces from stems and branches were colonized with *Fusarium* spp. We also found that over 40% of all sampled wood pieces were not colonized by any detectable fungi.

Collated levels of root, stem, and branch colonization by *Fusarium* spp. within sampled diseased trees are summarized in Table 3. Fourteen different *Fusarium* spp. were detected within the 52 sampled trees exhibiting wilt/dieback symptoms. Although most species were isolated infrequently, *F. oxysporum* and *F. solani* were fairly common. A total of 42% of all sampled wood pieces from diseased trees were colonized by *Fusarium* spp.

DISCUSSION

We found that *Fusarium* spp. are common residents of koa trees exhibiting wilt/dieback disease symptoms in Hawaii. Many different *Fusarium* spp. were detected, but the most common were *F. oxysporum* within roots and *F. solani* within stems and branches. We suspect that many of the other *Fusarium* spp. colonized wood tissues as non-pathogenic fungal endophytes and they probably do not adversely affect their hosts. Some of these species, such as *F. subglutinans* (Wollenw. & Reinking) and *F. semitectum* Berk. & Rav., are commonly found on koa seeds (James 2004; James and others 2006) and may have colonized seedlings following seed germination. Several of the others may have been introduced in other ways, such as on feeding insects and/or direct infection of tissues from either soil- or wind-borne inoculum.

Fusarium oxysporum was concentrated within koa root systems. Fungal isolates from this species complex were especially common on fine roots. We did not find necrosis or other disease symptoms on colonized roots. Based on pathogenicity tests conducted thus far, only a small percentage of *F. oxysporum* isolates from diseased trees were highly virulent on inoculated seedlings from susceptible seedlots (Dudley and others 2007a, 2007b). Unfortunately, highly-virulent isolates cannot be identified on the basis of morphological characteristics (Gordon and Martyn 1997; Kistler 1997). In some cases, they may be differentiated using special molecular markers (Bao and others 2002; Lori and others 2004; Roncero and others 2003). However, their ability to exhibit aggressiveness and elicit disease must be confirmed via inoculation tests.

Preliminary molecular genetic analyses of *F. oxysporum* populations associated with diseased koa trees indicated that populations are quite genetically diverse and that highly-virulent isolates may be clustered together, indicating genetic similarity (Yang and others 2007). Strictly non-pathogenic *F. oxysporum* isolates may likewise be clustered together within

phylogenetic trees. We are in the process of relating genetic clustering of selected *F. oxysporum* isolates with their ability to cause disease on koa seedlings. When this work is completed, we will have a clearer idea of the relationships between genetics and pathogenicity within *F. oxysporum* populations associated with diseased koa in Hawaii. Since strains of fungi within the *F. oxysporum* species complex vary in their ability to cause plant diseases (Gordon and Martyn 1997; Kistler 1997), it is no surprise that only a portion of the population is pathogenic. If molecular markers can be developed which can quickly differentiate highly-virulent strains, we will be in a much better position to quantify pathogenic strains in nature.

We also found fairly high levels of *F. solani* within diseased koa trees, especially in stem and branch tissues. This collaborates previous findings where this species commonly colonized the interior of koa trees infested with black twig borers (Daehler and Dudley 2002). We have only evaluated a few isolates of *F. solani* for their potential to cause wilt disease on koa seedlings (Dudley and others 2007b). It appears that some strains of this fungus may be moderately virulent on koa, at least under greenhouse inoculation conditions. It is possible that *F. solani* may contribute to disease severity by increasing plant stresses, thus making trees more susceptible to virulent, wilt-inducing isolates strains of *F. oxysporum*. However, much more work is needed to evaluate possible synergistic roles of different *Fusarium* spp. in disease severity.

This work increases the evidence that *F. oxysporum* is probably important in the etiology of wilt/dieback disease of *Acacia koa* trees. Strains of this fungus are usually present at relatively high levels within diseased trees, especially within root systems. Selected strains have exhibited high virulence in greenhouse inoculation tests on seedlings (Dudley and others 2007a). We have not yet systematically sampled koa trees without disease symptoms to compare levels of *Fusarium* colonization of non-diseased with diseased trees. Such work would improve our understanding of the effects of *Fusarium* colonization on production of disease.

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Table 1. Root colonization of *Acacia koa* trees exhibiting wilt/dieback symptoms with *Fusarium* spp.

Number of Trees Sampled	Fine Roots	Secondary Roots[1]	Tertiary Roots[2]	All Roots
	46	33	18	18
Pieces Sampled	763	600	430	1793
Fusarium spp.	Percent Colonization			
oxysporum	44.4	29.7	23.7	34.5
solani	16.2	8.3	6.5	11.3
graminearum	2.1	0	0.9	1.1
semitectum	0.6	1.0	1.2	0.9
lateritium	0.6	0.5	2.1	0.9
sambucinum	0.9	0.5	1.2	0.8
avenaceum	1.6	0	0	0.7
equiseti	1.2	0	0	0.5
subglutinans	0.8	0.3	0	0.4
proliferatum	0.9	0	0	0.4
acuminatum	0.8	0	0	0.3
sporotrichioides	0.1	0	0.5	0.2
All *Fusarium*	66.6	38.0	34.9	49.4
No Fungi	0	19.5	22.6	11.9

[1]Roots adjoining fine feeder roots

[2]Larger roots (usually a few cm in diameter) connected to secondary roots

Table 2. Stem and branch colonization of *Acacia koa* trees exhibiting wilt/dieback symptoms with *Fusarium* spp.

Number of Trees	Inner[1]	Outer[2]	All Samples
	43	43	43
Pieces Sampled	596	523	1119
Fusarium spp.	Percent Colonization		
solani	16.1	17.2	16.6
oxysporum	8.0	7.6	7.9
proliferatum	4.0	4.2	4.1
avenaceum	0.2	1.1	0.6
graminearum	0.5	0.2	0.4
subglutinans	0.3	0	0.2
acuminatum	0.3	0.2	0.2
sambucinum	0	0.4	0.2
chlamydosporum	0.2	0	0.1
semitectum	0.3	0	0.1
decemcelluare	0	0.2	0.1
sporotrichioides	0	0.2	0.1
All *Fusarium*	29.5	30.8	30.1
No Fungi	41.1	45.1	43.0

[1]Within the center of stems and branches, usually adjacent to a central core of green-black staining
[2]Adjacent to and inside the cambium near the bark

Table 3. Colonization of *Acacia koa* trees exhibiting wilt/dieback symptoms with *Fusarium* spp.

Number of Trees Sampled	52
Number of Wood Pieces Sampled	2912[1]
Fusarium Species	Percent Colonization
oxysporum	24.3
solani	13.5
proliferatum	1.8
graminearum	0.8
avenaceum	0.6
semitectum	0.6
sambucinum	0.6
lateritium	0.6
subglutinans	0.3
acuminatum	0.3
equiseti	0.3
sporotrichioides	0.1
chlamydosporum	0.03
decemcelluare	0.03
All *Fusarium* Species	42.0
No Fungi	23.9

[1]Includes pieces from roots, stems and branches

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