NURSERY DISEASE NOTES USDA FOREST SERVICE

NORTHERN REGION
FOREST HEALTH PROTECTION

No. 159 September 2004

FUSARIUM COLONIZATION OF SEEDS, SEEDPODS, AND DISEASED SEEDLINGS OF *ACACIA KOA* FROM HAWAII

R.L. James
Plant Pathologist

ABSTRACT

Nine different *Fusarium* species were isolated from *Acacia koa* seeds, seedpods, seedling stem and root tissues, rhizosphere soil and soil surrounding roots of diseased trees. *Fusarium oxysporum*, the cause of koa dieback and wilt, was not isolated from seeds or seedpods but was found most commonly on roots and soil near diseased koa seedlings. *Fusarium subglutinans* was the most common species isolated from seedpods, which had evidence of extensive insect predation. Other *Fusarium* spp. commonly isolated from seedlings and adjacent soil included *F. semitectum*, *F. equiseti*, and *F. solani*. Species isolated less frequently included *F. avenaceum*, *F. acuminatum*, *F. sambucinum*, and *F. sporotrichioides*. This work indicated that several *Fusarium* spp., in addition to *F. oxysporum*, may be common colonizers of diseased koa trees and seedlings. Further elucidation of their role in disease etiology is warranted.

INTRODUCTION

Koa (*Acacia koa* Gray) is an important native tree occurring on several of the Hawaiian Islands. This tree species not only provides important wildlife habitat (Rock 1974) but also produces high quality wood that is used to manufacture furniture, cabinets, paneling, picture framing, bowls, and carvings (Dudley 2004). In 1980, an important wilt disease on koa was initially described (Gardner 1980). The major associated pathogen, for which Koch's Postulates were

completed, was identified and assigned the taxon *Fusarium oxysporum* Schlecht. f.sp. *koae*. This pathogen was routinely isolated from stem and root tissues of wilted seedlings as well as commonly found on seeds of several varieties of *A. koa* and from a similar species (Formosan koa –*A. confusa* Merr.) (Gardner 1980). No other associated organisms, including those in the genus *Fusarium*, were described in this work.

Since its initial discovery, little work was done on this disease until the early 2000s. A stand-level analysis of the disease indicated that trees of all ages may become diseased under different stand conditions (Anderson et al. 2002). During this analysis, *F. oxysporum* f.sp. *koae* was the only fungus consistently isolated from diseased trees. Recovered isolates were also tested for pathogenicity, using a standard root dip inoculation procedure (Anderson et al. 2002; Gardner 1980). Very recent work has indicated that most isolates obtained from diseased trees belonged to a single mtDNA RFLP haplotype and a single vegetative compatibility group (VCG)(Anderson et al. 2004). Isolates from the same VCG were morphologically similar.

The only other reported fungus potentially associated with this disease was *F. solani* (Mart.) Appel & Wollenw. (Daehler and Dudley 2002). This species was frequently isolated from bodies of the Asian black twig borer (*Xylosandrus compactus*) as well as wood adjacent to attacked areas on koa trees. The fungus was not found outside infested portions of stems and branches and was not considered very virulent when tested for pathogenicity. It was therefore considered an incidental secondary colonizer of koa trees with *F. oxysporum* being the main pathogen.

Efforts to more fully understand koa dieback and wilt involves screening different cultivars or varieties of *A. koa* for genetic resistance to *F. oxysporum* f.sp. *koae* (Dudley 2004; Sniezko 2004). Hopefully this work will lead to identification of several high-performance families exhibiting significant resistance to this pathogen.

Although this disease was described nearly 25 years ago, only a few investigations of its pathology have been conducted. Therefore, additional work was needed, particularly clarification of the mycoflora that routinely exists on and within seeds, seedpods, and various tissues of seedlings displaying typical wilt disease symptoms. This report describes some of this work.

MATERIALS AND METHODS

Four sets of isolations were conducted:

1. Isolations from collected stored koa seed used in tree improvement and wilt resistance studies.
2. Isolations from seedpods and associated seed from a koa tree displaying typical dieback and wilt symptoms.
3. Isolations from six 3 month-old planted koa seedlings displaying typical dieback and wilt symptoms.
4. Isolations from three young koa seedlings displaying slight wilting symptoms including rhizosphere soil and soil surrounding seedling root systems.

Set 1:

Eight *A. koa* seedlots were assayed for fungal contamination on the outer seedcoat and colonization within the seed embryo. The seedlots were from the Hawaii Agriculture Research Center (HARC) Maunawili plantation (table 1).

One hundred seeds from each seedlot were assayed for fungal contamination and colonization; 80 of the seeds were aseptically placed directly on a selective agar medium designed specifically for *Fusarium oxysporum* (Komada 1975), even though other *Fusarium* species and some closely-related fungi, i.e., *Cylindrocarpon* spp., also readily grow on this medium (James et al. 1991). Some other common seed-contaminating fungi can also grow on this medium, although usually much slower (James 1999: James et al. 1996b). The remaining 20 seeds from each seedlot were surface sterilized in a 10% bleach (0.525% aqueous sodium hypochlorite) solution for 1 min (to eliminate most external contaminating fungi), rinsed in sterile water, and aseptically dissected with a sterile razor blade. Pieces of the seedcoat and internal embryo tissues were placed side by side on Komada's medium.

Plates with seeds were incubated for 10 days under diurnal cycles of cool, fluorescent light at about 24°C. After incubation, seeds were examined for presence of colonizing fungi. Most associated fungi were identified to genus directly from Komada's medium using the taxonomy of Barnett and Hunter (1998). Isolates suspected to be in the genus *Fusarium* were transferred, via single sporing, to potato dextrose agar (PDA) and carnation leaf agar (CLA)(Fisher et al. 1982) for identification using the taxonomy of Nelson et al. (1983). Growth on PDA was useful for elucidating overall colony morphology, mycelial and agar pigmentation, and extent of aerial mycelium and reversion to pionnotal colonies. CLA stimulated production of spores (macroconidia, microconidia, and chlamydospores), conidiophores, and sporodochia (Fisher et al. 1982). Percentages of sampled seeds contaminated (external seedcoat) or colonized (within seed embryo) by particular fungi were calculated. Germination of whole seeds was estimated after 10 days' incubation on agar; seeds were considered germinated if their radicle protruded through the seedcoat.

Set 2:

Isolations were made from 14 seedpods that had been collected from one wilted tree located at Waimano. Within the seedpods, most seeds had undergone extensive insect predation; only pieces of seed remained. Seedpods were aseptically dissected into pieces approximately 5 x 5 mm and placed directly onto Komada's medium. Seed pieces within seedpods were likewise placed directly onto this selective medium. No efforts were made to surface sterilize seedpod or seed tissues so that external contaminating fungi could be isolated. Plates with seedpod and seed pieces were incubated as described above. Procedures for identifying associated fungi were the same as described for seed assays. Percentages of sampled seedpod and seed pieces colonized by particular fungi were calculated.

Table 1. Characteristics of *Acacia koa* seedlots tested for fungal contamination and colonization.

Seedlot	HARC No.[1]	Identification[2]	Island/Location	Percent Survival[3]
1	OPR-5	98-1-OPR-5	Oahu	91.6
2	OPR-8	98-1-OPR-8	Oahu	50.0
3	KAHANA-c	98-1-Kahana-c	Oahu	75.0
4	ANAHOLA-2	92-2-Anahola-2	Kauai	16.6
5	F45P-2	98-5-F45P-2	Maui	45.8
6	KAPA-6	98-6-Kapa-6	Hawaii	16.6
7	KAPA-5	98-6-Kapa-5	Hawaii	12.5
8	93-313-9	93-313-9	Hamakua RS[4]	66.6

[1] HARC = Hawaii Agricultural Research Center
[2] First two numbers are the year of seed collection
[3] Percent survival of outplanted seedlings after 48 months on the Maunawili site on Oahu.
[4] Hamakua Research Station

Set 3:

Six seedlings with typical advanced wilt or dieback symptoms growing at the Dorcy Estate on Maui were assayed for fungal colonization on their stems and, in some cases, their roots. Assayed stem tissues were aseptically cut interior from the epidermis, surfaced sterilized, and incubated on Komada's medium. Roots were aseptically dissected into pieces approximately 5 mm in length, surface sterilized, and incubated on the selective medium. Procedures for identifying associated fungi were the same as in the other isolations. Percentages of sampled stem and root pieces colonized by particular fungi were calculated.

Set 4:

Extensive assays for colonizing fungi were conducted on three seedlings displaying slight wilting symptoms (slight chlorotic or reddening of foliage; no stem or branch dieback). These seedlings were also from the Dorcy estate on Maui. For each seedling, isolations were made from the main stem, primary (issuing from the main stem) and secondary (between primary branches and leaves) branches, roots (internal root tissues and fine roots), rhizosphere soil (adhering to roots after surrounding soil was removed), and soil surrounding roots. For all seedling tissue analyses, aseptic dissections were made, tissue pieces were surface sterilized and incubated on Komada's medium as previously described. Plates were incubated as before and procedures for identifying associated fungi were the same as for other isolations. Percentages of sampled tissue pieces colonized by particular fungi were calculated.

Procedures were conducted to determine approximate populations of selected fungi within rhizosphere and root-surrounding soil. Rhizosphere soil was assayed by washing seedling roots with 100 ml of sterile, distilled water and placing 2 ml of the resulting solution on plates of Komada's medium (three plates per seedling). The solution was

distributed uniformly over the agar surface; plates were incubated as before. There were too many resulting fungal colonies to count. Therefore, six of the most representative colonies per seedling were chosen for identification. Single spores of chosen colonies were obtained and transferred to PDA and CLA; resulting fungi were identified as described above.

Standard soil dilutions (Hildebrand and Dinkel 1988; James et al. 1990, 1996a; Stone et al. 1995) were conducted to estimate populations of *Fusarium* and *Trichoderma* spp. from soil surrounding roots of koa seedlings. Soil from each of the three samples was initially sieved (2-mm sieve) to remove rocks, pieces of organic matter, and soil aggregates. From each sample, an approximate 5-g subsample was oven-dried at about 100°C for at least 24 h until sample weight stabilized. Oven-dry weight was then calculated to provide a standard for sample comparison. For population assays, 0.05 g of field-moist soil was combined with 10 ml of 0.3% water agar (WA) and thoroughly mixed. One milliliter of solution was placed on each of three plates of Komada's medium and spread uniformly. *Trichoderma* propagules were also enumerated on Komada's medium which readily supports growth of this fungus unless the medium is amended with benomyl or lithium chloride (James et al. 1990, 1996a). Plates were incubated at least 7 days at about 24°C under diurnal cycles of cool, fluorescent light. *Fusarium* and *Trichoderma* colonies were identified by their morphology on the selective medium; populations were expressed as number of colony-forming units (cfu) per gram of oven-dried soil (it was assumed that each fungal colony originated from one propagule). Selected *Fusarium* isolates were transferred to PDA and CLA for identification as previously described. Ratios of *Trichoderma* to *Fusarium* populations were calculated for each seedling; these ratios might provide very rough estimates of potential disease suppressiveness of the soil since *Trichoderma* spp. are known antagonists of a wide range of soilborne plant pathogens, including *Fusarium* spp. (Papavizas 1985).

RESULTS

External seedcoat fungi were not detected on the vast majority of koa seed assayed (table 2). In three of 8 assayed seedlots, no fungi were detected on any seedcoats and in only one seedlot (7 – KAPA-5; Hawaii) were less than 90% of the sampled seed free from contaminating fungi. In this seedlot, no germination was detected after 10 days (table 2) and survival of outplanted seedlings was very low (table 1). The most common group of contaminating fungi on sampled seeds were in the genus *Penicillium*; *Aspergillus* and *Pestalotia* were found on only one seedlot (7 – KAPA-5; Hawaii).

Fusarium spp. were isolated at very low levels from three seedlots; the most contaminated seedlot was again number 7. Two *Fusarium* species were isolated: *F. avenaceum* (Fr.) Sacc. and *F. sambucinum* Fuckel; the former species was encountered more frequently.

Table 2. Fungal contamination of external seedcoats of *Acacia koa* from Maunalili seedlots[1].

Seedlot	*Fusarium*[2]	*Penicillium*	*Aspergillus*	*Pestalotia*	Clean[3]	Germ[4]
1	1.3	5.0	0	0	93.8	17.5
2	0	0	0	0	100.0	17.5
3	0	8.8	0	0	91.3	40.0
4	0	0	0	0	100.0	12.5
5	0	0	0	0	100.0	20.0
6	0	1.3	0	0	98.8	11.3
7	7.5	1.3	3.8	1.3	86.3	0
8	1.3	1.3	0	0	97.5	26.3
All Lots	1.3	2.2	0.5	0.2	95.9	18.1

[1] Values in table are percent of seeds assayed [80 per seedlot] with seedcoats colonized by appropriate fungus.
[2] All isolates were *Fusarium avenaceum* except one isolate in seedlot 7 which was *Fusarium sambucinum*.
[3] Percent of seeds without any fungal growth after 10 days' incubation.
[4] Percent of seeds starting to germinate [radicle protruding through the seedcoat] after 10 days' incubation on agar.

Fungal colonization of internal koa seed tissues was variable among the tested seedlots (table 3). *Penicillium* was again the major group of fungi isolated; *Aspergillus* was isolated from only one seedlot (1 – OPR-5; Oahu). This seedlot also had all of its assayed seeds colonized internally by *Penicillium* spp. Three of the assayed seedlots were without any detected fungal colonization of internal seed tissues; two of these (4 – ANAHOLA-2; Kauai and 5 –F45P-2; Maui) also had none of their sampled seed contaminated with seedcoat fungi (table 2). No *Fusarium* was isolated from internal koa seed tissues. *Fusarium oxysporum*, the cause of koa wilt (Anderson et al. 2002; Gardner 1980) was not isolated either from external seedcoats or within internal seed tissues.

Seedpods and pieces of seed within these seedpods were extensively colonized by *Fusarium* and *Penicillium* spp. (tables 4 and 5). Five different *Fusarium* species were isolated from seedpods and seed pieces: *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas, *F. semitectum* Berk. & Rav., *F. solani, F. sambucinum*, and *F sporotrichioides* Sherb. *Fusarium oxysporum*, the cause of koa wilt, was not isolated. Very high incidence of *F. subglutinans* was unexpected; these isolates were placed within the *Fusarium* section *Liseola* and designated as *F. subglutinans* because they produced microconidia on poly- and monophialides in false heads only (no microconidial chains), lacked chlamydospores, and produced purple pigments in PDA culture.

Isolations from the stems and roots of severely-wilted koa seedlings yielded fairly high levels of *Fusarium* (table 6). However, *F. oxysporum* was not isolated from two of the 6 sampled seedlings and found at fairly low levels in the others. The most common *Fusarium* species isolated from these seedlings was *F. equiseti* (Corda) Sacc.

Table 3. Internal fungal colonization of *Acacia koa* seeds from Maunalili seedlots[1].

Seedlot	*Penicillium*	*Aspergillus*	Clean[2]
1	100	5	0
2	20	0	80
3	40	0	60
4	0	0	100
5	0	0	100
6	65	0	35
7	5	0	95
8	0	0	100
All Lots	28.8	6.3	65.0

[1] Values in table are percent of assayed seed [20 per seedlot] colonized by appropriate fungus following dissection and exposure of embryo and endosperm.
[2] Percent of seeds without any fungal growth after 10 days' incubation.

Table 4. Fungal colonization of seedpods from a wilted *Acacia koa* tree.

Pod No.	No. Sampled[1]	Percentage Colonization[2]							
		FSUB	FSEM	FSOL	FSAM	FSPO	ALL FUS	PEN	TRI
1	19	57.9	5.3	5.3	0	5.3	73.7	68.4	5.3
2	15	66.7	0	0	0	0	66.7	100.0	6.7
3	17	23.5	0	0	0	5.9	29.4	100.0	5.9
4	18	55.5	0	0	0	0	55.5	72.2	5.5
5	12	8.3	0	8.3	8.3	0	25.0	83.3	0
6	10	20.0	10.0	0	0	0	30.0	100.0	20.0
7	15	20.0	6.7	6.7	6.7	0	40.0	93.3	0
8	13	53.8	7.7	0	0	0	61.5	92.3	7.7
9	17	94.1	0	0	0	0	94.1	35.3	5.9
10	17	94.1	0	0	0	0	94.1	76.5	0
11	14	64.3	0	7.1	0	0	71.4	100.0	0
12	14	50.0	0	0	0	0	50.0	78.6	0
13	15	20.0	0	0	0	0	20.0	93.3	6.7
14	14	11.1	0	0	0	0	11.1	94.4	11.1
Ave.	15.0	48.1	1.9	1.9	0.9	0.9	53.8	85.2	5.2

[1] Number of seedpod pieces (about 5 x 5 mm each) sampled.
[2] Percentage of sampled seedpod pieces colonized with appropriate fungus. Fungal abbreviations: FSUB = *Fusarium subglutinans*; FSEM = *Fusarium semitectum*; FSOL = *Fusarium solani*; FSAM = *Fusarium sambucinum*; FSPO = *Fusarium sporotrichioides*; ALL FUS = All *Fusarium* species; PEN = *Penicillium* spp.; TRI = *Trichoderma* spp.

Table 5. Fungal colonization of seed pieces from insect-predated seedpods from a wilted *Acacia koa* tree.

Pod No.	No. Sampled[1]	Percentage Colonization[2]							
		FSUB	FSEM	FSOL	FSAM	FSPO	ALL FUS	PEN	TRI
1	1	0	0	0	0	0	0	0	100.0
2	6	66.7	0	0	0	0	66.7	100.0	0
3	5	0	0	20.0	0	0	20.0	100.0	0
4	4	25.0	0	0	0	0	25.0	100.0	0
5	8	50.0	0	0	12.5	0	62.5	0	0
6	15	20.0	13.3	0	0	0	33.3	93.3	6.7
7	7 –	28.6	0	14.3	0	0	42.9	100.0	14.3
8	8	37.5	0	0	0	0	37.5	100.0	0
9	3	100.0	0	0	0	0	100.0	0	0
10	5	80.0	0	0	0	0	80.0	60.0	0
11	9	33.3	0	0	0	0	33.3	100.0	0
12	9	44.4	0	0	0	0	44.4	77.8	0
13	7	28.6	0	0	0	0	28.6	85.7	0
14	4	25.0	0	0	0	0	25.0	100.0	0
Ave.	6.5	37.4	2.2	2.2	1.1	0	42.9	80.2	3.3

[1] Number of seed pieces sampled per seedpod.
[2] Percentage of sampled seed pieces colonized with appropriate fungus. Fungal abbreviations: FSUB = *Fusarium subglutinans*; FSEM = *Fusarium semitectum*; FSOL = *Fusarium solani*; FSAM = *Fusarium sambucinum*; FSPO = *Fusarium sporotrichioides*; ALL FUS = All *Fusarium* species; PEN = *Penicillium* spp.; TRI = *Trichoderma* spp.

Table 6. *Fusarium* colonization of stems and roots of severely-wilted *Acacia koa* seedlings.

Seedling Number	Tissue Type	Pieces Sampled	Percent Colonization[1]				
			FEQU	FOXY	FSOL	FACU	ALL FUS
1	Stem	7	14.2	0	0	14.2	24.6
2	Stem	8	100.0	0	0	0	100.0
2	Roots	10	50.0	10.0	10.0	0	70.0
3	Stem	6	33.3	50.0	0	0	83.3
4	Stem	6	66.7	0	0	0	66.7
4	Roots	10	70.0	0	30.0	50.0	100.0
5	Stem	7	28.6	14.3	0	14.3	57.6
6	Stem	7	14.3	14.3	0	0	28.6
Averages	-	-	49.2	9.8	6.6	11.4	68.8

[1] Percent of sampled tissues pieces colonized with appropriate fungus. Fungal abbreviations: FEQU – *Fusarium equiseti*; FOXY – *Fusarium oxysporum*; FSOL – *Fusarium solani*; FACU – *Fusarium acuminatum*; ALL FUS – All *Fusarium* species.

Results of extensive isolations from stems, branches, and roots of three slightly-wilted koa seedlings are summarized in tables 7, 8, and 9, respectively. Internal stem tissues were not commonly colonized by fungi, although canker margins were more commonly colonized (table 7). *Fusarium oxysporum* was isolated very infrequently from internal stem tissues and more commonly from internal margins of stem cankers. Other *Fusarium* species isolated infrequently from stems included *F. semitectum, F. solani*, and *F. avenaceum*.

Colonization of primary branches (those coming from the stem) by fungi was less than colonization of secondary branches (those between primary branches and leaves)(table 8). *Fusarium oxysporum* was not isolated from primary branches and at very low levels from the secondary branches of only two seedlings. *Fusarium semitectum* was the most commonly-isolated species from both primary and secondary branches; *F. avenaceum* was also fairly common on secondary branches.

Fusarium oxysporum was most frequently isolated from seedling roots, especially the fine feeder roots (table 9). Other *Fusarium* root colonizers included *F. solani, F. equiseti*, and, in one instance, *F. subglutinans. Fusarium* spp. were isolated from all sampled fine roots and almost half of the internal root tissues sampled.

Fusarium spp. extensively colonized the rhizosphere of the three sampled seedlings. Levels were too high to be quantified using standard soil dilution procedures. *Fusarium oxysporum* was isolated from the rhizosphere of two of the seedlings (table 10); *F. solani* was more common, colonizing the rhizosphere of all three seedlings.

Very high levels of *Fusarium* were also detected in soil surrounding seedling roots (table 11). Populations averaged almost 5000 cfu/g of soil; this high level greatly exceeds threshold levels for nursery soils of about 1000 cfu/g, above which growers should expect disease losses (Hildebrand and Dinkel 1988; James et al. 1990, 1996a). Populations of *Trichoderma* spp., which have often characterized disease-suppressive soils and may act as natural biological control agents of *Fusarium* spp. (Papavizas 1985), where very low. Experience indicates that when *Fusarium* populations are high, corresponding *Trichoderma* populations are low (Datnoff et al. 1995; James et al. 1990, 1996a). This results in low *Trichoderma/Fusarium* ratios, which may indicate low potential disease suppressiveness.

The two most common *Fusarium* species colonizing soil were *F. equiseti* and *F. oxysporum* (table 12). Soil with high levels of *F. equiseti* had corresponding low populations of *F. oxysporum*, and vice versa. Other *Fusarium* species found less frequently included *F. solani, F. semitectum* and *F. avenaceum*.

Table 7. Fungal colonization of internal stem tissues of slightly-wilted *Acacia koa* seedlings[1].

Seedling Number	Healthy-Appearing Internal Stem Tissues [Non-Cankered]								
	None	FOXY	FSEM	FSOL	FAVE	ALL FUS	ASP	BOT	TRI
1	7	1	0	0	1	2	1	0	0
2	9	0	0	1	0	1	0	0	0
3	10	0	0	0	0	0	0	0	0
Percent[2]	86.7	3.3	0	3.3	3.3	10.0	3.3	0	0
	Internal Margins of Cankered Stem Tissues								
1	2	3	4	0	0	7	0	2	1
2	6	4	0	0	1	4	0	0	0
3	9	0	1	0	0	1	0	0	1
Percent[2]	56.7	23.3	16.7	0	3.3	40.0	0	6.7	6.7

[1] Values in table are numbers of sampled tissue pieces [10 per seedling] colonized with appropriate fungus. Fungal abbreviations: None – No isolated fungi; FOXY – *Fusarium oxysporum*; FSEM – *Fusarium semitectum*; FSOL – *Fusarium solani*; FAVE – *Fusarium avenaceum*; All FUS – All *Fusarium* species; ASP – *Aspergillus* spp.; BOT – *Botrytis cinerea*; TRI – *Trichoderma* spp.
[2] Percent of sampled pieces colonized with appropriate fungus.

Table 8. Fungal colonization of primary and secondary branches of slightly-wilted *Acacia koa* seedlings[1].

Seedling Number	Primary Branches[2]						
	None	FOXY	FSEM	FAVE	ALL FUS	ASP	BOT
1	10	0	0	0	0	0	0
2	8	0	0	0	0	1	1
3	5	0	5	0	5	0	0
Percent[4]	76.7	0	16.7	0	16.7	3.3	3.3
	Secondary Branches[3]						
1	4	0	6	0	6	0	0
2	0	3	0	10	10	0	1
3	0	1	10	4	10	0	0
Percent[4]	13.3	13.3	53.3	46.7	86.7	0	3.3

[1] Values in table are number of pieces sampled [10 per seedling] colonized by appropriate fungus. Fungal abbreviations: None – No Fungus Isolated; FOXY – *Fusarium oxysporum*; FSEM – *Fusarium semitectum*; FAVE – *Fusarium avenaceum*; ALL FUS – All *Fusarium* Species; ASP – *Aspergillus* spp.; BOT – *Botrytis cinerea*.
[2] Primary branches are from the main stem.
[3] Secondary branches are from the primary branches to the leaves.
[4] Percent of sampled pieces colonized with appropriate fungus.

Table 9. Fungal colonization of roots of slightly-wilted *Acacia koa* seedlings[1].

Seedling Number	Internal Root Tissues[2]					
	None	FOXY	FSOL	FEQU	FSUB	ALL FUS
1	5	3	1	1	0	5
2	5	5	0	0	0	5
3	6	2	3	0	1	4
Percent[4]	53.3	33.3	13.3	3.3	3.3	46.7
	Fine Roots[3]					
1	0	9	3	0	0	10
2	0	10	0	0	0	10
3	0	4	7	4	0	10
Percent[4]	0	76.7	33.3	13.3	0	100.0

[1] Values in table are number of pieces sampled [10 per seedling] colonized by appropriate fungus. Fungal abbreviations: None – No Fungus Isolated; FOXY – *Fusarium oxysporum;* FSOL – *Fusarium solani*; FEQU – *Fusarium equiseti*; FSUB – *Fusarium subglutinans*; ALL FUS – All *Fusarium* Species.
[2] Tissues within the main taproot.
[3] Fine [feeder] roots dissected into small pieces.
[4] Percent of sampled pieces colonized with appropriate fungus.

Table 10. *Fusarium* species isolated from rhizosphere soil on roots of slightly-wilted *Acacia koa* seedlings.

Seedling Number	Number of Isolates Examined	Percent of *Fusarium* Isolates[1]	
		FOXY	FSOL
1	6	16.7	83.3
2	6	66.7	33.3
3	6	0	100.0

[1] Fungal abbreviations: FOXY – *Fusarium oxysporum*; FSOL – *Fusarium solani*

Table 11. Populations of *Fusarium* and *Trichoderma* from soil surrounding roots of slightly-wilted *Acacia koa* seedlings.

Seedling Number	Populations [cfu/g of soil][1]			Number of *Fusarium* Isolates Examined
	Fusarium	*Trichoderma*	T/F Ratio[2]	
1	5733	276	0.048	249
2	4710	23	0.005	203
3	4297	0	0	178
Averages	4925	102	0.021	630

[1] Expressed as colony-forming units per gram of oven-dried soil.
[2] Ratio of *Trichoderma* to *Fusarium* populations; the higher the ratio, the more potential disease suppressive is the soil.

Table 12. *Fusarium* species isolated from soil surrounding roots of slightly-wilted *Acacia koa* seedlings.

Seedling Number	Percent of *Fusarium* Isolates[1]				
	FEQU	FOXY	FSOL	FSEM	FAVE
1	77.1	22.9	0	0	0
2	0	92.6	7.4	0	0
3	62.4	1.7	3.9	21.3	10.7

[1] Fungal abbreviations: FEQU – *Fusarium equiseti*; FOXY – *Fusarium oxysporum*; FSOL – *Fusarium solani;* FSEM – *Fusarium semitectum*; FAVE – *Fusarium avenaceum.*

DISCUSSION

Wilt caused by *F. oxysporum* f.sp. *koae* is an important disease limiting production of koa trees on several Hawaiian Islands. Previous work (Anderson et al. 2002; Gardner 1980) emphasized the important role and common presence of this fungal species on trees exhibiting wilt and dieback symptoms. The only other *Fusarium* species reported on damaged trees was *F. solani*, which was associated with attacks by the Asian black twig borer (Daehler and Dudley 2002). *Fusarium solani* was considered secondary and not important in wilt disease etiology.

Previous isolations from diseased koa trees were made onto standard, non-selective agar media (Anderson et al. 2002; Daehler and Dudley 2002; Gardner 1980). Probably the fastest growing fungi present were more readily isolated using this type of media. Other associated fungi, which grow slower on the isolation media, may have been overlooked (Hoff et al. 2004). Using a selective agar medium for *Fusarium* (Komada 1975) has been standard protocol in many investigations when trying to isolate these important pathogens (Hildebrand and Dinkel 1988; Hildebrand et al. 2004; James et al. 1990, 1996a, 1996b; Stone et al. 1995). Many common saprophytes are usually excluded. However, the medium is not so selective as to exclude all organisms besides *Fusarium.* For example, *Trichoderma* spp. and *Botrytis cinerea* grow quite well on the medium unless it is amended with benomyl or lithium chloride (Hansen et al. 1990). In addition, although the medium was initially formulated to enumerate populations of *Fusarium oxysporum* from soil (Komada 1975), most other *Fusarium* species grow well on the medium. Therefore, experience over many years has indicated that using this selective medium is very important when trying to isolate and quantify *Fusarium* spp. within plant tissues or soil (Hansen et al. 1990; James et al. 1991).

Nine different *Fusarium* species were isolated from diseased koa seedpods, seed pieces, seedlings, rhizosphere soil and soil adjacent to roots: *F. semitectum, F. sambucinum, F. sporotrichioides, F. equiseti, F. acuminatum, F. avenaceum, F. subglutinans, F. oxysporum* and *F. solani.* Some were frequently encountered, such as *F. subglutinans* on seedpods and seed pieces and *F. equiseti* on seedling stems, roots and within soil near diseased seedlings. Others were

isolated at lower levels. Many of these may be non-pathogens on koa and either secondary colonizers of diseased tissues or endophytes. However, it is possible that some of these fusaria may either contribute to disease etiology or help predispose hosts to infection by pathogens, such as *F. oxysporum* f.sp. *koae*. For example, endophytes in the genus *Fusarium* are known to affect disease severity caused by other pathogenic fungi (Narisawa et al. 2002; Stone et al. 2000).

All of these *Fusarium* species have previously been described as potential plant pathogens. For example, *F. semitectum* has been implicated in diseases of *Anigozanthos* spp. (Satou et al. 2001) and walnut (*Juglans*)(Belisario et al. 2002). *Fusarium avenaceum* may be an important pathogen on cereals (Kiecana et al. 2002) and several other crops, including lupin (Satyaprasad et al. 2000) and conifer seedlings (Asiegub et al. 1999). *Fusarium equiseti* may cause seed diseases (Aigbe et al. 1999) and diseases on pine (Dick and Dobbie 2002), but is usually considered a secondary invader rather than a primary plant pathogen (Francis and Burgess 1975; Gonzalez and Trevanthan 2000). *Fusarium sambucinum* is an important pathogen of potatoes (Beremand et al. 1991; Desjardins and Gardner 1991; Desjardins et al. 1993) and is frequently isolated from diseased conifer seedlings (Gordon 1959; James 1985). *Fusarium sporotrichioides* can cause diseases of several different hosts including water milfoil (Andrews and Hecht 1981), cereals (Chelkowski et al. 1989) and conifer seedlings (James 1985; Rathbun-Gravatt 1925). *Fusarium acuminatum* is a potential pathogen of cereals (Hill and Blunt 1974), Russian-olive seedlings (Hildebrand 1986) and conifer seedlings (James 1985, 1987). However, the most notorious potential pathogens of this group are *F. oxysporum* and *F. solani*.

Fusarium solani has a long history as an important pathogen on many crops, including trees (O'Donnell 2000). This species has been implicated in canker diseases of cottonwood (Boyer 1961), yellow-poplar (Dochinger and Seliskar 1962), sycamore (Pilotti et al. 2002) and sugar maple (Skelly and Wood 1966; Wood and Skelly 1964).

In several of the isolations reported here, *F. oxysporum* was not the most commonly-isolated *Fusarium* species. For example, *Fusarium oxysporum* was not isolated from any of the 8 sampled seedlots, although it has been reported as an important colonizer of koa seeds (Gardner 1980). Sampled seeds were often not colonized by any fungus and if fungi were present, they were considered saprophytes. Two *Fusarium* species (*F. avenaceum* and *F. sambucinum*) were isolated from the seedcoats of a very few sampled seeds; no *Fusarium* was isolated from internal seed tissues. *Fusarium oxysporum* was only isolated frequently from seedling roots, rhizosphere soil, and soil near roots.

All examined seedpods from a diseased tree had evidence of extensive insect predation. Most seeds had been destroyed and only remnants or pieces of seeds remained. Isolations from these seedpods and seed pieces yielded five different *Fusarium* species. The most common was identified as *F. subglutinans* based on morphological characteristics. This species has been implicated in several important plant diseases, the most important being pitch

canker disease of pines (Bonello et al. 2001; Enebak and Stanosz 2003; Gordon et al. 2001; Schmale and Gordon 2003; Storer et al. 1998; Viljoen et al. 1997) and disease of pineapple (Hidalgo et al. 1999). The pitch canker fungus was originally known as *F. subglutinans* f.sp. *pini*; recent taxonomic revision has resulted in renaming the pathogen *F. circinatum* Nirenberg & O'Donnell (Gordon et al. 2001). Pitch canker is intimately associated with insects. Twig beetles (*Pityophthorus*) (Hoover et al. 1996), _cone beetles (*Conophthorus*)(Hoover et al. 1995, 1996) and engraver beetles (*Ips*)(Fox et al. 1991) are known to vector the fungus and spread infection from tree to tree (Gordon et al. 2001). Therefore, it is possible that insects feeding on koa seedpods and/or seeds carried *F. subglutinans* within and possibly between trees.

Fusarium subglutinans is one of four species described in the section *Liseola* by Nelson et al. (1983). All species within this section have colony morphologies on PDA that are similar to each other and to *F. oxysporum*. Microscopic examinations are required to differentiate the various taxa. For example, if isolates produce chlamydospores they are not classified within the section *Liseola*. If they lack chlamydospores and produce microconidia on mono- and polyphialides in false heads only (no chains) they are, by definition, classified as *F. subglutinans* under Nelson's taxonomic scheme. This taxonomic system works well when only morphological characteristics are considered, but may not necessarily reflect actual speciation of different isolates.

In the past few years, molecular analyses have been used to characterize phylogeny of fungi in the genus *Fusarium* (Benyon et al. 2000; Guadet et al. 1989). Some of this work involved organisms within the section *Liseola* (*Gibberella fujikuroi* species complex) (O'Donnell et al.1998). In general, genetic analyses indicate that populations are usually more diverse than would be expected if fungi were classified based solely on morphological characteristics, and previously-accepted taxa often contain several cryptic species. For example, recent revision of the *Fusarium* section *Liseola* (O'Donnell et al. 1998) placed at least 36 species in this group, where previously only four were assigned based on morphological characteristics. Therefore, classifying isolates from koa seedpods and seed pieces as *F. subglutinans* must be considered tentative, at least until these isolates are genetically characterized.

Similar problems exist within organisms classified as *F. oxysporum*. This taxon is actually a group of morphologically similar species that may genetically differ from each other (Baayen et al. 2000; Di Pietro et al. 2003; Gordon and Martyn 1997; Katan 1999). Although no teleomorph has been found for *F. oxysporum*, wide genetic diversity still occurs among some clones of this species (Baayen et al. 2000; Gordon and Martyn 1997; Katan 1999). Therefore, organisms classified as *F. oxysporum*, based on morphological characteristics, may actually represent a range of different biological species.

The assignment of formae speciales names, such as f.sp. *koae*, are based on the pathogenicity of certain *F. oxysporum* isolates on specific hosts

(Armstrong and Armstrong 1975; Roncero et al. 2003). Therefore, isolates obtained from koa trees with wilt and dieback symptoms and capable of causing disease during controlled pathogenicity tests are classified as *F. oxysporum* f.sp. *koae*. Apparently, much of the population of this pathogen within Hawaii is fairly uniform genetically (Anderson et al. 2004); this may indicate that the population, at least on specific islands, could be clonal. Also, if the population is not genetically diverse, the pathogen may have been present in Hawaii for only a short time. Higher genetic variabililty would be expected from a population that had a longer time to mutate and diversify (Gordon and Martyn 1997). This may also indicate fairly recent introductions of the pathogen into Hawaii, perhaps only once. Further work will be needed to more fully understand the genetic characteristics of the *F. oxysporum* population associated with diseased koa trees.

Because pathogenicity tests were not conducted on the *F. oxysporum* isolates obtained from diseased seedlings or soil in the current evaluation, it is unknown if these isolates represent pathogens or are strictly saprophytic; saprophytic isolates often make up large portions of *F. oxysporum* populations in soil and on plants (Bao and Lazarovits 2001; Benhamou and Garand 2001; Larkin and Fravel 2002; Lori et al. 2004). Fortunately, recent molecular tests have been developed that help separate pathogenic from non-pathogenic populations of *F. oxysporum* (Bao et al. 2002; Di Pietro et al. 2003; Pasquali et al. 2003; Stewart et al. 2004). Molecular probes developed for pathogenic isolates may preclude the necessity for standard isolations and pathogenicity tests (Geiser et al. 2004; Li and Hartman 2003).

From these series of isolations it is evident that colonization of *Acacia koa* by a wide range of different *Fusarium* spp. may be common. Several species, in addition to *F. oxysporum* and *F. solani* that were previously described on koa (Anderson et al. 2002; Daehler and Dudley 2002; Gardner 1980), were commonly isolated. It is not known what, if any, the role these other species may play in dieback and wilt of koa seedlings and trees. However, since many of these fungi have a history of being important plant pathogens, at least on other hosts, further work to elucidate their role(s) in disease etiology is warranted.

LITERATURE CITED

Aigbe, S.O., B. Fawole and D.K. Berner. 1999. A cowpea seed rot disease caused by *Fusarium equiseti* identified in Nigeria. Plant Disease 83:964.

Anderson, R.C., T.C. Bentley and J.C. Correll 2004. Population diversity of *Fusarium oxysporum* f.sp. *koae*, the pathogen of Koa dieback in Hawaii. Phytopathology 94:S4 (Abstract).

Anderson, R.C., D.E. Gardner, C.C. Daehler and F.C. Meinzer. 2002. Dieback of *Acacia koa* in Hawaii: ecological and pathological characteristics of affected stands. Forest Ecology and Management 162:273-286.

Andrews, J.H. and E.P. Hecht. 1981. Evidence for pathogenicity of

Fusarium sporotrichioides to Eurasian water milfoil, *Myriophyllum spicatum*. Canadian Journal of Botany 59:1069-1077.

Armstrong, G.M. and J.K. Armstrong. 1975. Reflections on the wilt fusaria. Annual Review of Phytopathology 13:95-103.

Asiegbu, F.O., M. Johansson and J. Stenlid. 1999. Reactions of *Pinus sylvestris* (Scots pine) root tissues to the presence of mutualistic, saprotrophic and necrotrophic microorganisms. Journal of Phytopathology 147:257-264.

Baayen, R.P., K. O'Donnell, P.J.M. Bonants, E. Cigelnik, L.P.N.M. Kroon, E.J.A. Roebroeck and C. Waalwijk. 2000 Gene genealogies and AFLP analyses in the *Fusarium oxysporum* complex identify monophyletic and nonmonophyletic formae speciales causing wilt and rot disease. Phytopathology 90:891-900.

Bao, J.R., D.R. Fravel, N.R. O'Neill, G. Lazarovits and P. van Berkum. 2002. Genetic analysis of pathogenic and nonpathogenic *Fusarium oxysporum* from tomato plants. Canadian Journal of Botany.80:271-279.

Bao, J.R. and G. Lazarovits. 2001. Differential colonization of tomato roots by nonpathogenic and pathogenic *Fusarium oxysporum* strains may influence Fusarium wilt control. Phytopathology 91:449-456.

Barnett, H.L. and B.B. Hunter. 1998. Illustrated genera of imperfect fungi. The American Phytopathological Society Press, St. Paul, MN. 218p.

Belisario, A., M. Maccaroni and L. Corazza. 2002. Occurrence and etiology of brown apical necrosis on Persian (English) walnut fruit. Plant Disease 86:599-602.

Benhamou, N. and C. Garand. 2001. Cytological analysis of defense-related mechanisms induced in pea root tissues in response to colonization by nonpathogenic *Fusarium oxysporum* Fo47. Phytopathology 91:730-740.

Benyon, F.H.L., L.W. Burgess and P.J. Sharp. 2000. Molecular genetic investigations and reclassification of *Fusarium* species in sections *Fusarium* and *Roseum*. Mycological Research 104:1164-1174.

Beremand, M.N., A.E. Desjardins, T.M. Hohn and F.L. VanMiddlesworth. 1991. Survey of *Fusarium sambucinum* (*Gibberella pulicaris*) for mating type, trichothecene production, and other selected traits. Phytopathology 81:1452-1458.

Bonello, P., T.R. Gordon and A.J. Storer. 2001. Systemic induced resistance in Monterey pine. Forest Pathology 31:99-106.

Boyer, M.G. 1961. A *Fusarium* canker disease of *Populus deltoides*. Canadian Journal of Botany 39:1195-1204.

Chelkowski, J., M. Manka, H. Kwasna, A. Visconti and P. Golinski. 1989. *Fusarium sporotrichioides, Fusarium tricinctum* and *Fusarium poae* - cultural characteristics, toxinogenicity and pathogenicity towards cereals. Journal of Phytopathology 124:155-161.

Daehler, C.C. and N. Dudley. 2002. Impact of the black twig borer, an introduced insect pest, on *Acacia koa* in the Hawaiian Islands. Micronesica 6:35-53.

Datnoff, L.E., S. Nemec and K. Pernezny. 1995. Biological control of Fusarium crown and root rot of tomato in Florida using *Trichoderma harzianum* and *Glomus intraradices*. Biological Control 5:427-431.

Desjardins, A.E., E.A. Christ-Harned, S.P. McCormick and G.A. Secor. 1993. Population structure and genetic analysis of field resistance to thiabendazole in *Gibberella pulicaris* from potato tubers. Phytopathology 83:164-170.

Desjardins, A.E. and H.W. Gardner. 1991. Virulence of *Gibberella pulicaris* on potato tubers and its relationship to a gene for rishitin metabolism. Phytopathology 81:429-435.

Dick, M.A. and K. Dobbie. 2002. Species of *Fusarium* on *Pinus radiata* in New Zealand. New Zealand Plant Protection 55:58-62.

Di Pietro, A., M.P. Madrid, Z. Carcuel, J. Delgado-Jarana and M.I.G. Roncero. 2003. *Fusarium oxysporum*: exploring the molecular arsenal of a vascular wilt fungus. Molecular Plant Pathology 4:315-325.

Dochinger, L.S. and C.E. Seliskar. 1962. *Fusarium* bark canker of yellow-poplar. Phytopathology 52:8.

Dudley, N. 2004. 2-year growth performance of selected *Acacia koa* progeny at Maunawili, Oahu. Hawaii Agriculture Research Center. Unpublished. 20p.

Enebak, S.A. and G.R. Stanosz. 2003. Responses of conifer species of the Great Lakes region of North America to inoculation with the pitch canker fungus *Fusarium circinatum*. Forest Pathology 33:333-338.

Fox, J.W., D.L. Wood and C.S. Koehler. 1990. Distribution and abundance of engraver beetles (Scolytidae: Ips spp.) on Monterey pines infected with pitch canker. Canadian Entomologist 123:1355-1367.

Fisher, N.L., L.W. Burgess, T.A. Toussoun and P.E. Nelson. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. Phytopathology 72:151-153.

Francis, R.G. and L.W. Burgess. 1975. Surveys of Fusaria and other fungi associated with stalk rot of maize in eastern Australia. Australian Journal of Agricultural Research 26:801-807.

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

Geiser, D.M., M. del Mar Jimenez-Gasco, S. Kang, I. Makalowska, N. Veeraraghavan, T.J. Ward, N. Zhang, G.A. Kuldau and K. O'Donnell. 2004. FUSARIUM-ID v. 1.0: A DNA sequence database for identifying *Fusarium*. European Journal of Plant Pathology 110:473-479.

Gonzalez, M.S. and L.E. Trevathan. 2000. Identity and pathogenicity of fungi associated with root and crown rot of soft red winter wheat grown on the upper Coastal Plain land resource area of Mississippi. Journal of Phytopathology 148:77-85.

Gordon, T.R. and R.D. Martyn. 1997. The evolutionary biology of *Fusarium oxysporum*. Annual Review of Phytopathology 35:111-128.

Gordon, T.R., A.J. Storer and D.L. Wood. 2001. The pitch canker epidemic in California. Plant Disease 85:1128-1139.

Gordon, W.L. 1959. The occurrence of *Fusarium* species in Canada. VI. Taxonomy and geographic distribution of *Fusarium* species on plants, insects, and fungi. Canadian Journal of Botany 37:257-290.

Guadet, J., J. Julien, J.-F. Lafay and Y. Brygoo. 1989. Phylogeny of some *Fusarium* species, as determined by large-subunit rRNA sequence comparison. Molecular Biology and Evolution 6:227-242.

Hansen, E.M., D.D. Myrold and P.B. Hamm. 1990. Effects of soil fumigation and cover crops on potential pathogens, microbial activity, nitrogen availability, and seedling quality in conifer nurseries. Phytopathology 80:698-704.

Hidalgo, O.B., R. Santos, R.T. Tussel, A. Pires de Matos, R.S. Cabral, M. Arzola and M.C. Perez. 1999. Phytotoxicity of *Fusarium subglutinans* culture filtrates on in vitro plantlets and calli of resistant and susceptible pineapple (*Ananas comosus*). Plant Pathology 48:756-758.

Hildebrand, D.M. 1986. *Fusarium acuminatum* and a tip blight of Russian-olive. USDA Forest Service, Rocky Mountain Region, Forest Pest Management. Biological Evaluation R2-86-1. 3p.

Hildebrand, D.M. and G.B. Dinkel. 1988. Evaluation of methyl bromide, Basamid granular, and solar heating for pre-plant pest control for fall-sown eastern redcedar at Bessey Nursery. USDA Forest Service, Rocky Mountain Region, Timber, Forest Pest, and Cooperative Forestry Management. Technical Report R2-41. 13p.

Hildebrand, D.M., J.K. Stone, R.L. James and S.J. Frankel. 2004. Alternatives to preplant soil fumigation for western forest nurseries. USDA Forest Service, Pacific Northwest Research Station. General Technical Report PNW-GTR-608. 27p.

Hill, J.P. and D.L. Blunt. 1994. Wheat seedling response to root infection by *Cochliobolus sativus* and *Fusarium acuminatum*. Plant Disease 78:1150-1152.

Hoff, J.A., N.B. Klopfenstein, G.I. McDonald, J.R. Tonn, M.-S. Kim, P.J. Zambino, P.F. Hessbury, J.D. Rogers, T.L. Peever and L.M. Carris. 2004. Fungal endophytes in woody roots of Douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine. (*Pinus ponderosa*). Forest Pathology 34:255-271.

Hoover, K., D.L. Wood, J.W. Fox and W.E. Bros. 1995. Quantitative and seasonal association of the pitch canker fungus, *Fusarium subglutinans* f.sp. *pini*, with *Conophothorus radiatae* (Coleoptera: Scolytidae) and *Ernobius punctulatus* (Coleoptera: Anobiidae) which infest *Pinus radiata*. Canadian Entomologist 127:79-91.

Hoover, K., D.L. Wood, A.J. Storer, J.W. Fox and W.E. Bros. 1996. Transmission of the pitch canker fungus, *Fusarium subglutinans* f.sp. *pini* to Monterey pine, *Pinus radiata*, by cone- and twig-infesting beetles. Canadian Entomologist 128:981-994.

James, R.L. 1985. *Fusarium* associated with seedborne diseases of ponderosa pine seedlings at the Montana State Nursery, Missoula. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes No. 23. 5p.

James, R.L. 1987. Containerized grand fir seedling mortality - North Woods Nursery, Elk River, ID. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes No. 58. 4p.

James, R.L. 1999. Ponderosa pine seed fungal contamination: effects of stratification and sterilizing treatments. USDA Forest Service, Northern Region, Forest Health Protection. Report 99-3. 11p.

James, R.L., S. Metzger and C.J. Gilligan. 1990. Effects of soil fumigation on conifer seedling production at the USDA Forest Service Nursery, Coeur d'alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Report 90-11. 18p.

James, R.L., R.K. Dumroese and D.L. Wenny. 1991. *Fusarium* diseases of conifer seedlings. *In*: Sutherland, J.R. and S.G. Glover (eds.). Proceedings of the First Meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries). Forestry Canada. Pacific and Yukon Region. Information Report BC-X-331. pp. 181-190.

James, R.L., D.S. Page-Dumroese, S.K. Kimball and S. Omi. 1996a. Effects of *Brassica* cover crop, organic amendment, fallowing, and soil fumigation on production of bareroot Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 96-6. 10p.

James, R.L., R.K. Dumroese and D.L. Wenny. 1996b. Western larch seed contaminating fungi and treatments to reduce infection and improve germination. USDA Forest Service, Northern Region, Forest Health Protection. Report 96-7. 14p.

Katan, T. 1999. Current status of vegetative compatibility groups in *Fusarium oxysporum* 27: 51-64.

Kiecana, I., E. Mielniczuk, Z. Kaczmarek, M. Kostecki and P. Golinski. 2002. Scab response and moniliformin accumulation in kernels of oat genotypes inoculated with *Fusarium avenaceum* in Poland. European Journal of Plant Pathology 108:245-251.

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Review of Plant Protection Research (Japan) 8:114-125.

Larkin, R.P. and D.R. Fravel. 2002. Effects of varying environmental conditions on biological control of Fusarium wilt of tomato by nonpathogenic *Fusarium* spp. Phytopathology 92:1160-1166.

Li, S. and G.L. Hartman. 2003. Molecular detection of *Fusarium solani* f.sp. *glycines* in soybean roots and soil. Plant Pathology 52:74-83.

Lori, G., V. Edel-Hermann, N. Gautheron and C. Alabouvette. 2004. Genetic diversity of pathogenic and nonpathogenic populations of *Fusarium oxysporum* isolated from carnation fields in Argentina. Phytopathology 94:661-668.

Narisawa, K., H. Kawamata, R.S. Currah and T. Hashiba. 2002. Suppression of Verticillium wilt in eggplant by some fungal root endophytes. European Journal of Plant Pathology 108:103-109.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas. 1983. *Fusarium* species: an illustrated manual for identification. The Pennsylvania State University Press, University Park. 193p.

O'Donnell, K.O. 2000. Molecular phylogeny of the *Nectria haematococca-Fusarium solani* species complex. Mycologia 92:919-938.

O'Donnell, K., E. Cigelnik and H.I. Nirenberg. 1998. Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. Mycologia 90:465-493.

Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. Annual Review of Phytopathology 23:23-54.

Pasquali, M., A. Acquadro, V. Balmas, Q. Migheli, A. Garibaldi and M.L. Guillino. 2003. RAPD characterization of *Fusarium oxysporum* isolates pathogenic on *Argyranthemum frutescens*. Journal of Phytopathology 151:30-35.

Pilotti, M., V. Ponzio and E. Motta. 2002. Disorders of *Platanus* x *acerifolia* in Italy associated with *Fusarium solani*. Forest Pathology 32:249-264.

Rathbun-Gravatt, A. 1925. Direct inoculation of coniferous stems with damping-off fungi. Journal of Agricultural Research 30:327-339.

Rock, J.F. 1974. The indigenous trees of the Hawaiian Islands. Pacific Tropical Botanical Gardens, Lawaii, Hawaii.

Roncero, M.I.G., C. Hera, M. Ruiz-Rubio, F.I. Garcia Maceira, M.P. Madrid, Z. Caracuel, F. Calero, J. Delgado-Jarana, R. Roldan-Rodriguez, A.L. Martinez-Rocha, C. Velasco, J. Roa, M. Martin-Urdiroz, D. Cordoba, and A. Di Pietro. 2003. *Fusarium* as a model for studying virulence in soilborne plant pathogens. Physiological and Molecular Plant Pathology 62:87-98.

Satou, M., M. Ichinoe, F. Fukumoto, N. Tezuka and S. Horiuchi. 2001. Fusarium blight of Kangaroo Paw (Anigozanthos spp.) caused by *Fusarium chlamydosporum* and *Fusarium semitectum*. Journal of Phytopathology 149:203-206.

Satyaprasad, K., G.L. Bateman and E. Ward. 2000. Comparisons of isolates of *Fusarium avenaceum* from white lupin and other crops by pathogenicity tests, DNA analyses and vegetative compatibility tests. Journal of Phytopathology 148:211-219.

Schmale, D.G. III and T.R. Gordon. 2003. Variation in susceptibility to pitch canker disease, caused by *Fusarium circinatum*, in native stands of *Pinus muricata*. Plant Pathology 52:720-725.

Skelly, J.M. and F.A. Wood. 1966. The occurrence and etiology of an annual canker of sugar maple in Pennsylvania. Canadian Journal of Botany 44:1401-1411.

Sniezko, R.A. 2004. Potential for selecting for genetic resistance to *Fusarium oxysporum* f.sp. koae (koa wilt pathogen) in *Acacia koa* (koa) for conservation, restoration, and utilization in Hawaii. USDA Forest Service, Pacific Northwest Region, Dorena Genetic Resource Center, Cottage Grove, OR. Trip Report (8/24/2003 – 9/3/2003). 13p.

Stewart, J.E., M.-S. Kim, R.L. James, R.K. Dumroese and N.B. Klopfenstein. 2004. Molecular characterization of *Fusarium oxysporum* from tree nurseries: Tools for early detection of pathogens. Phytopathology 94:S99 (Abstract).

Stone, J.K., C.W. Bacon and J.F. White, Jr. 2000. An overview of endophytic microbes: endophytism defined. *In*: Bacon, C.W. and J.F. White, Jr. (eds.). Microbial Endophytes. Macel Dekker, Inc., New York. pp. 3-29.

Stone, J.K., D.M. Hildebrand, R.L. James, S.M. Frankel and D.S. Germandt. 1995. Alternatives to methyl bromide for control of soil-borne diseases in bare root forest nurseries. In: Proceedings: Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions. November 6-8, 1995, San Diego, CA. Methyl Bromide Alternatives Outreach, US Environmental Protection Agency and US Department of Agriculture. pp. 77-1 – 77-4.

Storer, A.J., T.R. Gordon and S.L. Clark. 1998. Association of the pitch canker fungus, *Fusarium subglutinans* f.sp. *pini*, with Monterey pine seeds and seedlings in California. Plant Pathology 47:649-656.

Viljoen, A., W.F.O. Marasas, M.J. Wingfield and C.D. Viljoen. 1997. Characterization of *Fusarium subglutinans* f.sp. *pini* causing root disease of *Pinus patula* seedlings in South Africa. Mycological Research 101:437-445.

Wood, F.A. and J.M. Skelly. 1964. The etiology of an annual canker on maple. Phytopathology 54:269-272.

R.L. James is Plant Pathologist, USDA Forest Service, Northern Region, Forest Health Protection. Address: USDA Forest Service, 3815 Schreiber Way, Coeur d'Alene, ID 83814; email rjames@fs.fed.us.