Acacia koa Seedling Wilt Caused by *Fusarium oxysporum* f. sp. *koae*, f. sp. nov.

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ABSTRACT

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A wilt disease was observed among koa (*Acacia koa*) seedlings in Hawaii. *Fusarium oxysporum* was consistently isolated from root and stem tissue of these plants, as well as from wilted *A. confusa* and *A. koaia* seedlings. This fungus also may be responsible for, or contribute to, the premature decline and death of older koa trees established within Hawaii Volcanoes National Park. High wilt percentages among plants grown in a pathogen-free growth medium indicate that the fungus is seedborne. Seed disinfestation procedures did not reduce disease incidence. A new forma specialis, *Fusarium oxysporum* f. sp. *koae*, f. sp. nov., is proposed for this pathogen.

Koa (*Acacia koa* Gray) not only is a highly valued timber resource in Hawaii but, being a species native to the Hawaiian Islands, it occupies a critical position in native forest ecosystems. Within Hawaii Volcanoes National Park (HVNP), *A. Koa* var. *hawaiiensis* Rock (the variety native to the island of Hawaii) is the second most abundant tree species, and is the dominant tree at elevations above 1,250 m.

A wilt disease of koa first was observed on seedlings in the HVNP greenhouse (8). Characteristics included a rather sudden, irreversible shoot wilt and death which apparently was not caused by root system degeneration. Vascular discoloration was not evident in seedling roots or stems, but under humid conditions external fungal mycelium was visible on stems of severely wilted plants.

Isolation procedures consistently yielded a *Fusarium* referable to *F. oxysporum* (Schlecht.) emend. Snyd. & Hans. (4,16). Preliminary studies (8) indicated that the fungus was seedborne since the disease occurred with equal frequency among noninoculated seedlings in natural field soil, steamed soil, a commercial soil preparation, or new vermiculite. Isolation of *F. oxysporum* directly from seeds further supported this conclusion.

Although *F. oxysporum* is generally regarded as a soil inhabitant where it may survive saprophytically for extensive periods (4), reports of seedborne wilt fusaria in other plants are numerous (3–6,10–14).

Further studies of the koa wilt disease are reported here, and a forma specialis designation for the pathogen is proposed.

MATERIALS AND METHODS

Sources of seed. The majority of seeds used in the following tests was collected directly by the investigator and assistants from apparently healthy mature *A. koa* var. *hawaiiensis* trees established within HVNP or on the slopes of Mauna Kea. Seeds of Formosan koa (*A. confusa* Merr.) and false koa (*Leucaena leucocephala* [Lam.] de Wit) were likewise collected directly from trees established at lower elevations within HVNP. Seeds of *A. koa* var. *hawaiiensis* from other locations, and of other *A. koa* varieties (native to other islands), as well as of other *Acacia* spp., were obtained from J. M. Brewbaker and D. J. McKenna of the University of Hawaii, and from G. Clarke of the Hawaii State Forestry Division. Seeds of *A. koaia* Hbd. originating from the *Acacia koaia* Tree Sanctuary on the island of Hawaii were obtained from a limited collection of rare plant seeds maintained at HVNP.

Isolation of the pathogen. Roots and stems of wilted seedlings were surface disinfested for 2 min in 0.5% NaOCl, and sections were plated on acidified 2% water agar (WA). Throughout the present study this general procedure routinely was followed to confirm presence of the pathogen in association with wilt symptoms in seedlings. Fungus isolates used for reinoculation were cultured from single spores (7) on potato dextrose agar (PDA) or were mass transferred to PDA or acidified PDA.

Pathogenicity tests. Previous experience indicated the necessity for scarification of koa seeds to facilitate germination. This was accomplished by soaking them in hot (95 C) water as it cooled slowly to room temperature. To avoid possible effects of this treatment on the fungus, a small opening was made through the seed coats of non-heat-treated seeds as an alternate scarification method. Seeds were germinated in a steamed soil-cinder mix or in new vermiculite. Seedlings were root-dip inoculated in water suspensions of approximately 10^6 microconidia per milliliter. Controls were dipped similarly in sterile water.

Inoculated seedlings were transplanted either into soil mix or vermiculite, or were cultured hydroponically in tap water alone or in water amended with Stern's Miracle-Gro (Stern's Nurseries, Inc., Geneva, NY 14456) at a rate of 0.5 g/ L. All plants were maintained in a Freas Model 818 incubator (Precision Scientific Co., Chicago, IL 60647) under daylight fluorescent lighting (approximately 6,000 lux) 16 hr/day. The growth medium temperature was monitored with thermometers placed at the root zone, and was maintained at 27–28 C (2). Although results obtained in initial tests of hydroponic culture appeared equal to those obtained with the other culture methods, it was discontinued in later tests since Toole (15) questioned its validity in similar earlier experiments with mimosa (*Albizzia julibrissin* Durazz.) seedlings.

Inoculation also was attempted by growing seedlings in vermiculite amended with *F. oxysporum*-colonized rice or by pouring conidial suspensions into the growth medium around established seedlings.

The pathogen was reisolated from wilted seedlings on WA as described above. Freehand sections of newly infested stems also were prepared for direct microscopic examination.

Isolation from seeds. To elucidate the seedborne nature of the koa wilt pathogen, various disinfestation procedures were

followed with *A. koa* var. *hawaiiensis* or *A. confusa* seeds. Samples of the former (20 or 100 seeds per sample) were shaken in 0.5% NaOCl with a Wrist Action Shaker (Burrell Corp., Pittsburg, PA 15219) for progressively increasing 5 or 30 min periods up to 30 min or 4 hr, respectively. Different seed samples were used for each exposure time. Controls were treated similarly with water only. The seeds were mechanically scarified and plated on WA, on PDA, or were sown in vermiculite and maintained at 27–28 C.

In a further effort to eliminate surface-borne fungi, 100 *A. koa* var. *hawaiiensis* seeds were passed individually through an alcohol burner flame such that the seed coats were uniformly seared. These seeds and nontreated controls were likewise sown in vermiculite at 27–28 C.

Mechanically scarified seeds (100 seeds per treatment) of both of the above species were allowed to imbibe water for several hours to facilitate seed coat removal. Seed coats were plated separately from internal portions on WA or PDA. Intact seeds also were mechanically cracked or broken open to expose internal tissues and the seeds were plated.

Although the above disinfestation procedures were expected to eliminate surface-borne *F. oxysporum,* eradication of possible internally-borne fungi also was attempted. Lots of 20 mechanically scarified or nonscarifed *A. koa* var. *hawaiiensis* seeds were immersed in hot water (90 C) for 15 sec, 30 sec, 1 min, and 3 min periods up to 20 min. Seeds were also immersed in 70 C water for 30 min or in 50 C water for 1 hr. Heat treated seeds and nontreated controls were sown in vermiculite and maintained at 27–28 C.

Haware et al (9) reported the effectiveness of particular fungicides in eradication of internally-borne *F. oxysporum* from chickpea seed. In a modification of their procedure, *A. koa* var. *hawaiiensis* seeds (200 seeds per lot) were soaked in an aqueous suspension of 2.0 g/L of 50% benomyl and 1.35 g/L of 75% thiram for 1, 4, 16, or 48 hr. All treated seeds and controls were sown in vermiculite and grown at 27–28 C.

Isolation from older trees. Symptoms indicative of a vascular wilt disease leading to the decline and eventual death of several koa saplings and mature trees established within HVNP have been under observation by the investigator. Following bark removal, branch and root samples were sectioned, surface disinfested in 0.5% NaOCl, and plated on WA in an effort to isolate the pathogen.

RESULTS

The pathogen. *Fusarium oxysporum* (4,16) was isolated from all diseased plant tissue throughout this study and cultured on PDA. Colonies exposed to daylight fluorescent lighting at room temperature or incubated at 28 C under similar lighting conditions were typically floccose and ranged in color from vinaceous to deep purple shades with light purple being the most common. Production of microconidia was prolific in all cultures and macroconidia also were abundant. Conidia were produced in false heads. Chlamydospores were predominantly smooth walled and singly, although occasionally doubly, produced in terminal or intercalary positions (16). Chlamydospores developed profusely on submerged mycelium surrounding infected plant tissue sections in older WA cultures.

Diseased seedlings. Throughout the experiments, more than 1,800 wilted inoculated and noninoculated seedlings were observed. The majority of these subsequently were sectioned and plated for isolation or reisolation of the pathogen. Except for the small number of plants that yielded bacteria, *F. oxysporum* was recovered from at least one stem or root section of each seedling. Most plants produced fungal growth from the cut ends of either several or all sections, which indicated systemic distribution.

Microscopic examination of stem sections frequently revealed the presence of hyphae and conidia associated with vascular tissue. External development of *F. oxysporum* mycelium on the stems of severely wilted seedlings sometimes became evident, although root systems of diseased seedlings appeared healthy until shoot death was complete (Fig. 1).

Isolation from older trees. Disease symptoms among mature trees included progressive yellowing and irreversible wilting of the crown in general or of major branches individually, often accompanied by vascular discoloration in branch tissue as defoliation proceeded. Complete death of an entire tree or a major branch usually occurred 2–6 mo after symptoms became evident.

F. oxysporum was isolated from both root and branch tissues of dead or dying trees. This recovery was less consistent than that observed from diseased seedlings, however, and it was complicated by the presence of the other fungi. Evidence of *F. oxysporum* as the

TABLE 1. Disease development caused by *Fusarium oxysporum* f. sp. *koae,* f. sp. nov. among naturally-infected[a] and root-dip inoculated seedlings of koa and related species

Host species	Total seedlings[b]		Total seedlings wilted			
	Inoculated	Noninoculated	Inoculated	Wilt (%)	Noninoculated	Wilt (%)
Acacia Koa	375	330	266	71	112	34
A. confusa	107	105	63	60	32	31
A. koaia	41	41	35	85	17	42
A. simplicifolia	13	13	3	23	6	46
Leucaena leucocephala	100	100	0	...	0	...

[a] Growing from pathogen-infested seed.
[b] All seedlings were grown under 16 hr/day illumination and 27–28 C growth medium temperature.

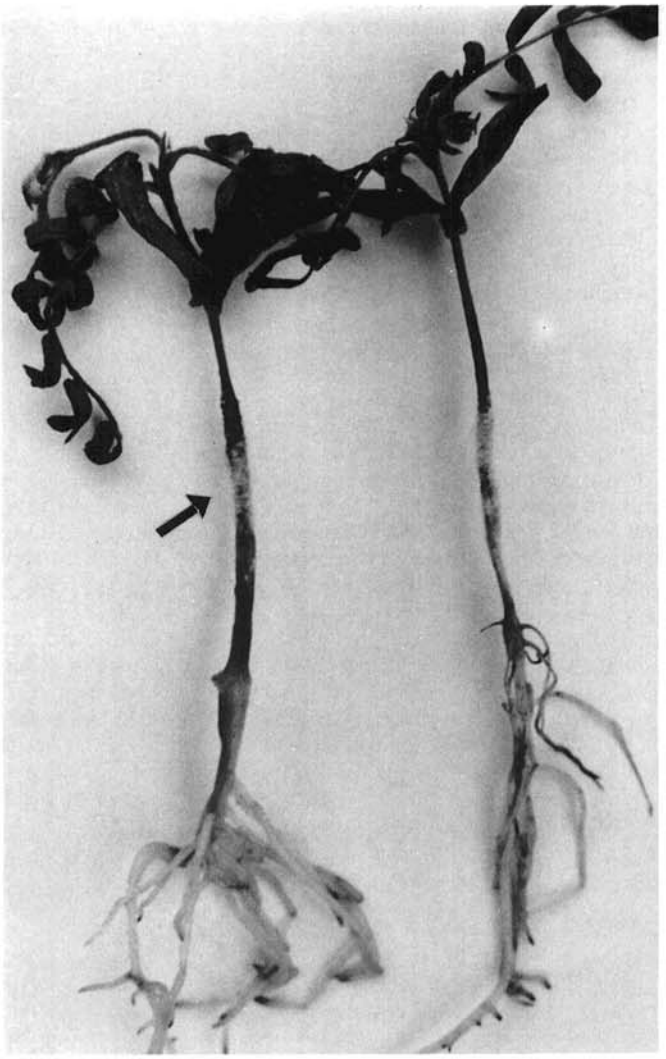

Fig. 1. External growth of *Fusarium oxysporum* f. sp. *koae,* f. sp. nov., on stems of wilted *Acacia koa* var. *hawaiiensis* seedlings.

single cause of decline of mature trees was, therefore, somewhat less conclusive. PDA cultures of *F. oxysporum* isolated from the above sources produced colonies with coloration, morphology, macro- and microconidia, and chlamydospores characteristic of this species.

Inoculation tests. Of the seedling inoculation methods used, the root-dip procedure was the most reliable and satisfactory, and was used in the majority of inoculation tests. *F. oxysporum* was so frequently associated with seed collected from *A. koa* var. *hawaiiensis* and related hosts that a pathogen-free seed source of a susceptible plant species was not identified in this study. In most cases, however, inoculation resulted in an increase in disease incidence in susceptible species as compared with noninoculated seedlings (Table 1). The wilt organism was not associated with seeds or seedlings of false koa and all seedlings of this species remained healthy following inoculation. In addition to species listed in Table 1, a limited number of seedlings of *A. heterophylla* (Lam.) Willd. and of *A. melanoxylon* R. Br. (Australian blackwood) were tested with no resulting symptoms whether inoculated or noninoculated.

Isolates of *F. oxysporum* obtained from all sources in this study, including those from *Acacia* spp. other than *A. koa,* were morphologically similar to each other in every respect. Relative susceptibility among hosts was difficult to determine, however, by the limited supply of *A. koaia* seed and inavailability of pathogen-free seed.

Isolation from seeds. Direct isolation from seeds plated on WA was relatively more difficult than was isolation by other isolation procedures. *F. oxysporum* was isolated from approximately 9% of 300 *A. koa* var. *hawaiiensis* and *A. confusa* seeds plated. The fungus grew from seed coats of intact nonsurface-disinfested seeds as well as from cotyledon tissue of seeds whose internal portions had been exposed.

Of 100 intact seeds treated with 0.5% NaOCl for 4 hr (the maximum exposure time) prior to plating, 48 germinated and 20 of these developed severe wilt symptoms 22–30 days following emergence. Thirty-one of the nontreated controls wilted.

Germination rates of *A. koa* var. *hawaiiensis* seeds declined linearly from 66% among 200 control seeds to 13% following 8 min of immersion in 90 C water. No corresponding reduction in disease incidence was evident, however, 24 days after emergence. Thirty-one and 33% of the control and heat-treated seedlings wilted, respectively. *Fusarium*-colonized seed tissue and wilted seedlings of the 70- and 50-C treatments also were observed.

Germination among fire-treated seeds was totally inhibited. Approximately 30% of the ungerminated seeds nevertheless became heavily colonized with *F. oxysporum* when placed in the growth medium at 27–28 C. Similar extensive colonization of ungerminated seeds occurred commonly throughout the hot water and NaOCl treatments, and was often more pronounced on treated seeds and seed parts than on controls. As with seeds plated directly on WA, fungal growth was evident from internal parts as well as seed coats.

Seed treatment with the benomyl-thiram mixture effectively limited *F. oxysporum* colonization of seeds in the growth medium. Two seeds became colonized following the 1 hr exposure and the fungus was not observed following the 4-, 16-, or 48-hr treatments. However, seedling wilt was evident among each of these treatments 22 days following emergence and resulted in approximately 30% seedling mortality. No correlation was noted between fungicide exposure times and wilt reduction.

DISCUSSION

The disease described in this study is characterized by severe, permanent wilting of the leaves as the primary symptom (rather than root rot or rots of other plant parts). Vascular discoloration was also evident among mature trees in advanced stages of decline. *F. oxysporum* was consistently isolated from serial stem and root sections of diseased seedlings and confirmed in pure culture. The fungus was observed by direct microscopic examination (1) to be associated with vascular tissue.

Failure to eliminate the fungus from seeds with NaOCl, hot water, or exposure to fire indicates that the pathogen is internally seedborne. This conclusion is supported by direct isolation from internal tissue. Colonization of seed coats may have resulted from emergence of internally-borne *F. oxysporum,* or, in nontreated seeds, the fungus may be present on the seed surface as well. Although hot water treatments severely reduced seed germinability without eliminating the pathogen, further trials of exposure time-temperature combinations may provide an effective treatment procedure. Likewise, a more thorough investigation considering several variables is necessary before conclusions regarding fungicide effectiveness can be drawn.

The apparent rate of natural infestation was consistently high among seeds of *A. koa* from various locations, and among those of *A. confusa,* and *A. koaia.* Although spread of inoculum through the growth medium may have accounted for some of the observed wilt, the uniformity of symptom development among test seedlings suggested simultaneous infection rather than a pattern of successive disease development. Considering its apparent prevalence and the number of seed sources from which the pathogen was isolated in the present study, it is particularly surprising that koa wilt was not previously reported.

A possible explanation may involve the incubation temperatures which were presumably optimum for disease development in this study. Even though wilt was initially detected under more variable greenhouse conditions, the disease was found only in rare instances. The disease was not detected in frequent subsequent observations of greenhouse-grown koa seedlings from the same seed sources demonstrated to be colonized by *F. oxysporum* when the rooting medium temperature was maintained at 27–28 C in a growth chamber. Greenhouse temperatures typically ranged from 10 to 24 C over 24 hr, with a daily mean of approximately 16 C. The infrequency of symptom expression among koa stands within the principal study area (HVNP) may also be accounted for by the lack of temperatures conducive for disease development in nature.

Based upon information gained in this study, the pathogenic *F. oxysporum* isolates from *A. koa* sources and from other *Acacia* spp. appear to represent a single organism. The forma specialis designation *Fusarium oxysporum* (Schlecht.) emend. Snyd. & Hans. f. sp. *koae,* f. sp. nov., is proposed for this fungus.

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