Investigating koa wilt in Hawai‘i

examining *Acacia koa* seeds and seedpods for *Fusarium* species

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ABSTRACT

We sampled *Acacia koa* A. Gray (Fabaceae) seeds and seedpods from 4 of the Hawaiian Islands (Big Island, Kaua'i, O'ahu, and Maui) for colonization by and contamination with *Fusarium* spp. (Hyphomycetes). The vast majority of healthy-appearing seeds from storage were not colonized by *Fusarium*. Stored seeds with superficial fungal mycelium, however, were extensively contaminated by *Fusarium*. Nearly 80% of the sampled seeds from forest trees with koa wilt disease symptoms had evidence of insect predation. More than 70% of the insect-predated seeds were contaminated by *Fusarium*; about 60% of healthy-appearing seeds from diseased forest trees were also contaminated. Seedpods were commonly colonized by the same *Fusarium* species that contaminated seeds. Thirteen different *Fusarium* spp. were isolated from koa seeds and seedpods. Most species were found at low levels, although *F. semitectum*, *F. subglutinans*, and *F. solani* were frequently isolated. *Fusarium oxysporum*, the putative cause of koa wilt/dieback disease, was isolated very rarely from either seeds or seed coats. Ecological significance and potential disease roles of *Fusarium* contaminating koa seeds need to be determined.

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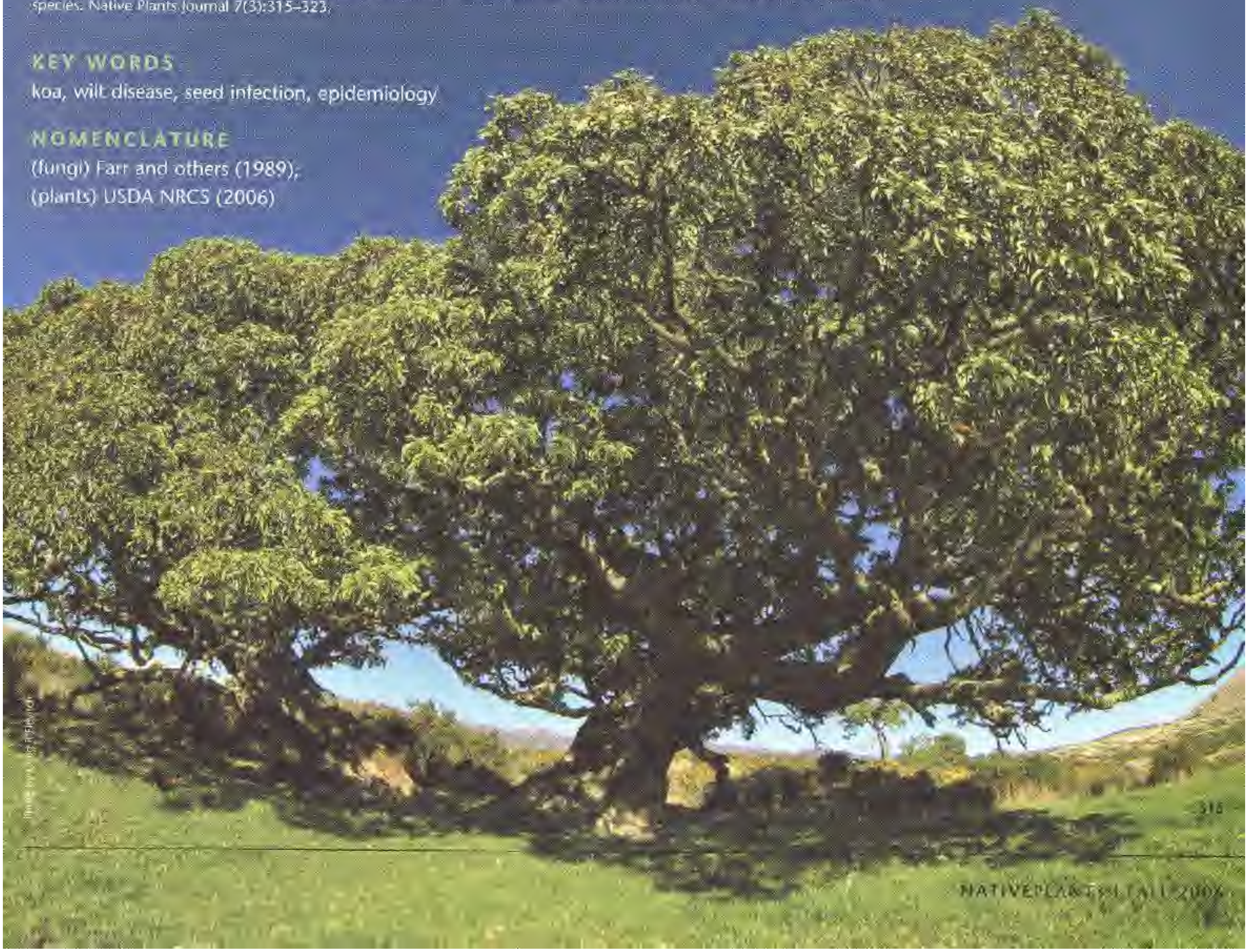
KEY WORDS
koa, wilt disease, seed infection, epidemiology

NOMENCLATURE
(fungi) Farr and others (1989);
(plants) USDA NRCS (2006)



Acacia koa A. Gray (Fabaceae) is an important tree species in Hawai`i from both an ecological and economic standpoint. This native species grows extremely fast on high-quality sites on most Hawaiian Islands. In recent years, koa has been extensively damaged by a wilt/dieback disease putatively caused by the fungus *Fusarium oxysporum* Schlechtend.:Fr. f.sp. *koae* D.E. Gardner (Hyphomycetes) (Gardner 1980; Anderson and others 2002). This disease is widespread, particularly on the 4 major Hawaiian Islands of 0`ahu, Kaua'i, Maui, and Hawaii (Big Island). Trees of all ages, from seedlings to overmature, are affected. Damage is particularly severe at elevations below 610 m (2000 ft) (Anderson and others 2002).

Infection of A. *koa* by *F. oxysporum* may result in systemic colonization of the host by the pathogen (Gardner 1980; Anderson and others 2002). Such colonization could result in the fungus infecting flowers and the resulting seeds. Although seed infection by this pathogen has been reported (Gardner 1980), quantitative information on levels of seed infection is not available.

Recent investigations (James 2004) indicated that several different species of *Fusarium* in addition to *F. oxysporum* may be found on A. *koa* plants exhibiting typical wilt/dieback symptoms. Although not all *Fusarium* isolates from diseased plants may be pathogens, we have found that some species other than *F. oxysporum* may either elicit disease symptoms or be capable of reducing tree growth. Therefore, it is important to know which *Fusarium* spp. routinely colonize koa seeds, their relative abundance, and how seeds might affect spread of potential pathogens.

MATERIALS AND METHODS

Seeds were sampled from either bulk storage or from collections made from planted or natural *Acacia koa* trees displaying wilt/dieback disease symptoms. Stored seeds either appeared healthy and were ready for sowing at nurseries (8 seedlots; 80 seeds sampled/lot) (Table 1) or had superficial fungal mycelial growth that was evident on seed coats (1 seedlot [Piha I; 83 seeds sampled) (Table 2). Seeds from trees exhibiting wilt/dieback disease symptoms (Gardner 1980) were collected from 6 locations either on (Oahu, the Big Island, or Kauai; these samples were divided into 3 categories: obvious insect-predated seeds, healthy-appearing (non-insect-predated) seeds, and pieces of seedpods that enclosed seeds. Sample sizes varied considerably among the 6 samples. A total of 656 *Acacia koa* insect-predated seeds were sampled (Table 3), whereas only 178 healthy-appearing seeds could be sampled (Table 4) because they comprised a minority of the samples. Seedpod samples from the 6 locations comprised 847 pieces (Table 5).

Seeds were aseptically placed directly on a selective agar medium for *Fusarium* and closely related fungi (Komada 1975). They were not surface sterilized because one of the goals of the evaluation was to elucidate extent of surface contamination by *Fusarium* spp. Usually 10 seeds were placed on each plate of selective medium. Seedpods were aseptically dissected into pieces about 5 to 8 mm in length and width. Randomly selected pieces were surface sterilized in a 10% bleach solution (0.525% aqueous sodium hypochlorite; 1 part standard household bleach in 10 parts water), rinsed in sterile, distilled water, and placed on the selective medium. All plates were incubated under diurnal cycles of cool, fluorescent light at about 24 °C (75 °F) for 7 to 10 days. Single spores of selected isolates were transferred onto potato dextrose agar and carnation leaf agar (Fisher and others 1982) for identification using the taxonomy of Nelson and others (1983). Percentages of sampled seeds and seedpod pieces colonized with each *Fusarium* spp. were calculated.

RESULTS AND DISCUSSION

Healthy-appearing seeds from storage were mostly free of fungal colonization or seed coat contamination (Table 1). Slightly more than 1% of the sampled seeds were contaminated by *Fusarium;* all isolates were identified as *F. avenaceum (Fr.:Fr.)* Sacc. (Hyphomycetes). Other fungi found infrequently either on seed coats or within seed embryos included *Penicillium*, *Aspergillus*, and *Pestalotia* (not identified to species). All of these fungi were likely saprophytes and would probably not adversely affect either seed germination or seedling establishment.

The one seedlot from storage with evident superficial fungal mycelial growth was extensively colonized by *Fusarium* spp. (Table 2). Nearly 80% of the sampled seeds were infected. Five different *Fusarium* species were isolated from this seedlot; by far the most common species was *F. semitectum* Berk. & Ravenel (Hyphomycetes).

We found a wide range of *Fusarium* (13 different species) on insect-predated or healthy-appearing *Acacia koa* seeds obtained from diseased trees on several sites on 0`ahu, the Big Island, and Kaua`i (Tables 3 and 4). Similar *Fusarium* species also readily colonized seedpods at the sampled sites (Table 5). Several *Fusarium* spp., such as *F. semitectum*, *F. subglutinans* (Wollenw. & Reinking) P.E. Nelson, T.A. Toussoun & Marasas, F *solani* (Mart.) Sacc., and *F. avenaceum*, were isolated most frequently. Most other species were isolated at low levels, including *F. oxysporum*, the putative cause of koa wilt/dieback disease. All of the fungi listed were contaminants located externally on seed coats or colonized seedpod tissues.

Fusarium oxysporum has previously been suspected of being transmitted on contaminated seeds, which might account for spread of the wilt/dieback disease throughout the state of Hawai`i (Gardner 1980). However, we found this particular *Fusarium* species only infre-

TABLE 1

Colonization of healthy-appearing Acacia koa *seeds from storage with selected fungi.*[z]

Seedlot	Island location	Percentage *Fusarium*[y]	Percentage clean[x]	Other fungi
OPR-5	O'ahu	1.2	93.8	None
OPR-8	O'ahu	0	100.0	None
Kahana-c	O'ahu	0	91.3	*Penicillium*
Anahola-2	Kaua'i	0	100.0	None
F45P-2	Maui	0	100.0	None
Kapa-6	Big Island	0	98.8	*Penicillium*
Kapa-5	Big Island	7.5	86.3	*Penicillium* *Aspergillus* *Pestalotia*
93-313-9	Big Island	1.3	97.5	*Penicillium*
All lots	—	1.25	95.9	—

z All sampled seeds were stored and ready for sowing in nurseries; 80 seeds were sampled per seedlot; 20 seeds from each seedlot were dissected to determine fungal colonization within the embryo—most were not colonized by fungi (*Penicillium* was found infrequently).
y All isolates were *F. avenaceum*.
x Percentage of seeds without any fungi detected on seed coats.

TABLE 2

Contamination of Acacia koa *stored seeds with superficial fungal mycelium with* Fusarium *species.*[z]

***Fusarium* species**	**Percentage of sampled seeds colonized** [z]
semitectum	55.4
sambucinum	12.0
solani	7.2
acuminatum	2.4
subglutinans	1.2
All *Fusarium* species	78.3

[z] Total number of seeds sampled = 83.

quently on seeds or seedpods from wilt/dieback diseased trees. Occurrence of *F. oxysporum* was much less common on seeds than several other *Fusarium* species. Therefore, at this time we cannot confirm that either pathogenic or nonpathogenic strains of *F. oxysporum* are being readily distributed on infested *Acacia koa* seeds.

We have recently found that koa seedlings being grown in nurseries may have roots extensively colonized by *F. oxysporum,* even without infected seedlings displaying typical wilt disease symptoms. Therefore, it is possible that this fungal species is being distributed on infected nursery stock. Sources of *F. oxysporum* colonizing nursery stock are currently unknown and require investigation.

Most sampled seeds collected from koa trees had evidence of insect predation. There were few non-predated seeds within sampled seedpods. It is possible that insects may be involved in vectoring fungi, especially *Fusarium* spp., among infested seedpods. We did not identify the insect species associated with seed predation; how many and which species are primarily involved are unknown. Further work is needed to identify the

TABLE 3

Percentage of sampled Acacia koa *insect-predated seeds from the Big Island of Hawai'i and Kaua'i colonized by* Fusarium *species.*

***Fusarium* species** [z]	Sample location						
	Waimano O'ahu (%)	Volcano National Park (%)	Miscellaneous Kaua'i (%)	Hamakua Research Station—Big Island (%)	Miscellaneous Big Island Miscellaneous Kaua'i (%)	Opaeula Ridge —O'ahu (%)	All samples (%)
acuminatum				5.0			0.8
avenaceum		12.5		17.0	3.9		4.3
equiseti				3.0			0.5
graminearum				13.0			2.0
lateritium					12.6	0.5	4.6
oxysporum				11.0		3.9	2.7
poae		18.8					0.5
proliferatum				12.0			1.8
sambucinum	1.1						0.1
semitectum	2.2		89.7	7.0		29.4	14.8
solani	2.2				13.5	43.3	16.9
sporotrichioides					10.0		3.5
subglutinans	37.8				40.9		19.5
All *Fusarium*	43.3	31.3	89.7	66.0	80.9	76.7	71.5
Number of seeds sampled	90	16	39	100	230	180	656

[z] Authorities: *Fusarium acuminatum* Ellis & Everh.; *F. avenaceum* (Fr.:Fr.) Sacc.; *F. equiseti* (Corda) Sacc.; *F. graminearum* Schwabe; *F. lateritium* Nees:Fr.; *F. oxysporum* Schlechtend.:Fr.; *F. poae* (Peck) Wollenweb.; *F. proliferatum* (T. Matsushima) Nirenberg; *F. sambucinum* Fuckel; *F. semitectum* Berk. & Ravenel; *F. solani* (Mart.) Sacc.; *F. sporotrichioides* Sherb.; *F. subglutinans* (Wollenweb. & Reinking) P.E. Nelson, T.A. Toussoun & Marasas. All species are Hyphomycetes; some have teleomorphs in the genera *Gibberella* and *Nectria*.

insects involved and confirm their potential roles in vectoring seed- and seedpod-colonizing fungi.

It is possible that some seeds become colonized by *Fusarium* spp. by processes other than insect predation. Many healthy, non-predated seeds collected from diseased trees were also colonized by these fungi. They may have become contaminated from seedpod-colonizing fungi or perhaps they were exposed to *Fusarium* spp. during the process of seed development. Whatever the reason, more than 60% of the healthy seeds sampled from diseased trees were infected by *Fusarium* (Table 4), whereas very few healthy-appearing seeds sampled from storage were infected with *Fusarium* (Table 1).

Potential roles of seed-contaminating *Fusarium* spp. in eliciting diseases of *Acacia koa* are unknown. We suspect that most of these fungi are saprophytes and not capable of causing diseases. Two possible exceptions are *F. oxysporum* and *solani*. We have frequently isolated *F. solani* from diseased trees, as have Daehler and Dudley (2002), particularly within the interior root and stem wood. *Fusariurn solani* has also been associated with infestation of *Acacia koa* by the black twig borer (*Xylosandrus compactus* Eichhoff (Coleoptera: Scolytidae]) (Daehler and Dudley 2002) and may be frequently vectored by these insects.

It is interesting that relatively high levels of 2 *Fusariurn* species, *F. semitec-tum* and *F. subglutinans*, were consistently found at several locations on both seeds and seedpods. *Fusarium semitecturn* is mostly a tropical species (Nelson and others 1983; Jimenez and others 1997; Satou and others 2001) that produces powerful toxins (Abbas and others 1995; Logrieco and others 1998, 2002). It has been associated with diseases of several plants including *Anigozanthus* (kangaroo paw) in Australia (Satou and others 2001), potatoes (Bokshi and others 2003), bananas (Jimenez and others 1997), and Juglans (walnut) (Belisario and others 2002). *Fusarium semitectum* has been detected on seeds of several different plants including *Dalbergia nigra* (Dhingra and others 2003), *Anadenanthera macrocarpa* (Dhingra and others 2002), and melons (Shanda and others 1995), as well as on maize (Pitt and others 1993; Owolade and others 2001) and sorghum grain (Onyike and Nelson 1992). We have isolated this fungal species from diseased koa seedlings and trees, although not as frequently as some other *Fusarium* species. We tested one isolate of *F. semitectum* for pathogenicity on koa seedlings in a greenhouse test (the isolate was not from seeds or seed coats) and found that it induced seedling mortality and was moderately virulent when compared with some other *Fusarium* species.

Fusarium subglutinans was also frequently isolated from koa seeds and seedpods. This species, which is a mem-

Figure 1. Size and morphological variation of different seedlots of *Acacia koa* seeds.
Photo by NS Dudley

TABLE 4

Percentage of healthy-appearing (non-insect-predated) Acacia koa *seeds from the Big Island of Hawai'i and Kaua'i colonized by* Fusarium *species.*

Fusarium species[Z]	Sample location				
	Miscellaneous Kaua'i (%)	Piha—Big Island (%)	Hamakua Research Station—Big Island (%)	Miscellaneous Big Island Miscellaneous Kaua'i (%)	All samples (%)
acuminatum		2.4			1.1
avenaceum			16.1	8.3	6.7
graminearum			8.9		2.8
lateritium				2.8	0.6
oxysporum			1.8		0.6
sambucinum		12.0			5.6
semitectum	100.0	55.4	17.9		33.1
solani		7.2		8.3	5.1
sporotrichioides				8.3	1.7
subglutinans		1.2		13.9	3.4
All *Fusarium*	100.0	73.3	44.6	41.7	60.7
Number of seeds sampled	3	83	56	36	178

[Z] Authorities: *Fusarium acuminatum* Ellis & Everh.; *F. avenaceum* (Fr.:Fr.) Sacc.; *F. graminearum* Schwabe; *F. lateritium* Nees:Fr.; *F. oxysporum* Schlechtend.:Fr.; *F. sambucinum* Fuckel; *F. semitectum* Berk. & Ravenel; *F. solani* (Mart.) Sacc.; *F. sporotrichioides* Sherb.; *F. subglutinans* (Wollenweb. & Reinking) P.E. Nelson, T.A. Toussoun & Marasas. All species are Hyphomycetes; some have teleomorphs in the genera *Gibberella* and *Nectria*.

her of the *Fusarium* section *Liseola,* has recently undergone taxonomic revision (Nirenberg and O'Donnell 1998; O'Donnell and others 1998; Britz and others 1999). Therefore, our isolates may actually belong to different species, although they were initially classified as *F subglutinans* based on the morphological characteristics outlined by Nelson and others (1983). For example, strains originally classified as either *F moniliforme* var. *subglutinans* (Barrows-Broaddus and others 1985) or *F. subglutinans* f.sp. *pini* (Britz and others 1999), which cause pitch canker disease of conifers in relatively warm areas (Barrows-Broaddus and others 1985; Dwinell and others 1985; Britz and others 1999; Gordon and others 2001), are now called F *circinatum* Nirenberg & O'Donnell emend. Britz, Coutinho, Wingfield & Marasas. This fungus is commonly vectored by and closely associated with insects that attack susceptible trees. The disease is primarily restricted to warmer climates in the southeastern US, along the California coast, and in Haiti, Mexico, Japan, and South Africa (Viljoen and others 1995; Britz and others 1999; Gordon and others 2001). We recently evaluated 2 *F subglutinans* isolates from infested seeds for pathogenic potential on *Acacia koa* seedlings. One of the isolates was moderately virulent and the other was nonpathogenic under the conditions of our greenhouse test. Additional work is needed to determine the importance of *F. subglutinans* as a seed-contaminating fungus, the role of insects in possibly vectoring this species, how genetically diverse this species is within Hawaii, and the phylogenetic relationship of Hawaiian isolates with other fungal species within the *Fusarium* section *Liseola,* especially F *circinatum*.

We isolated a new species of *Fusarium* from koa seedpods that we have not previously encountered (Table 5). This species was identified as F *sterilihyphosum* Britz, Marasas & Wingfield on the basis of genetic analysis (O'Donnell 2005). It has been described only once before, associated with mango malformation in South Africa (Britz and others 2002). *Fusarium sterilihyphosum* is morphologically similar to F *subglutinans* but is differentiated primarily by production of definitive sterile coiled hyphae (Britz and others 2002).

In conclusion, we have found that *Fusarium* spp. are very common on *Acacia koa* seeds and seedpods from forest trees. Most seeds found on either planted or natural koa trees in Hawaiian forests are insect predated; these insects may be important in vectoring *Fusarium* associated with seeds. Much more work is needed to answer salient questions regarding the importance and potential of *Fusarium* on koa seeds in Hawaii.

TABLE 5

Colonization of Acacia koa *seedpods from the Big Island of Hawai'i and Kaua'i by* Fusarium *species.*

Fusarium species[Z]	Sample location: Waimano O'ahu (%)	Volcano National Park (%)	Miscellaneous Kaua'i (%)	Hamakua Research Station— Big Island (%)	Miscellaneous Big Island Miscellaneous Kaua'i (%)	Opaeula Ridge Big Island (%)	All samples (%)
acuminatum			8.0				0.9
avenaceum		47.3	8.0	75.0	13.9		19.5
graminearum				6.0	9.3	0.9	3.2
lateritium					26.0		6.6
oxysporum						1.8	0.2
proliferatum		1.8					0.2
sambucinum	0.9		11.0		1.9		2.0
semitectum	1.9		98.0		3.7	34.3	17.0
solani	1.9			3.0	13.9	36.1	9.0
sporotrichioides	0.9						0.2
sterilihyphosum				2.0			0.2
subglutinans	47.2			3.0	20.5		17.5
All *Fusarium*	52.8	49.1	100.0	86.0	80.0	72.2	71.2
Number of seedpod pieces sampled	214	110	100	100	215	108	847

[Z] Authorities: *Fusarium acuminatum* Ellis & Everh.; *F. avenaceum* (Fr.:Fr.) Sacc.; *F. graminearum* Schwabe; *F. lateritium* Nees:Fr.; *F. oxysporum* Schlechtend.:Fr.; *F. proliferatum* (T. Matsushima) Nirenberg; *F. sambucinum* Fuckel; *F. semitectum* Berk. & Ravenel; *F. solani* (Mart.) Sacc.; *F. sporotrichioides* Sherb.; *F. sterilihyphosum* Britz, Marasas & Wingfield; *F. subglutinans* (Wollenweb. & Reinking) P.E. Nelson, T.A. Toussoun & Marasas. All species are Hyphomycetes; some have teleomorphs in the genera *Gibberella* and *Nectria*.

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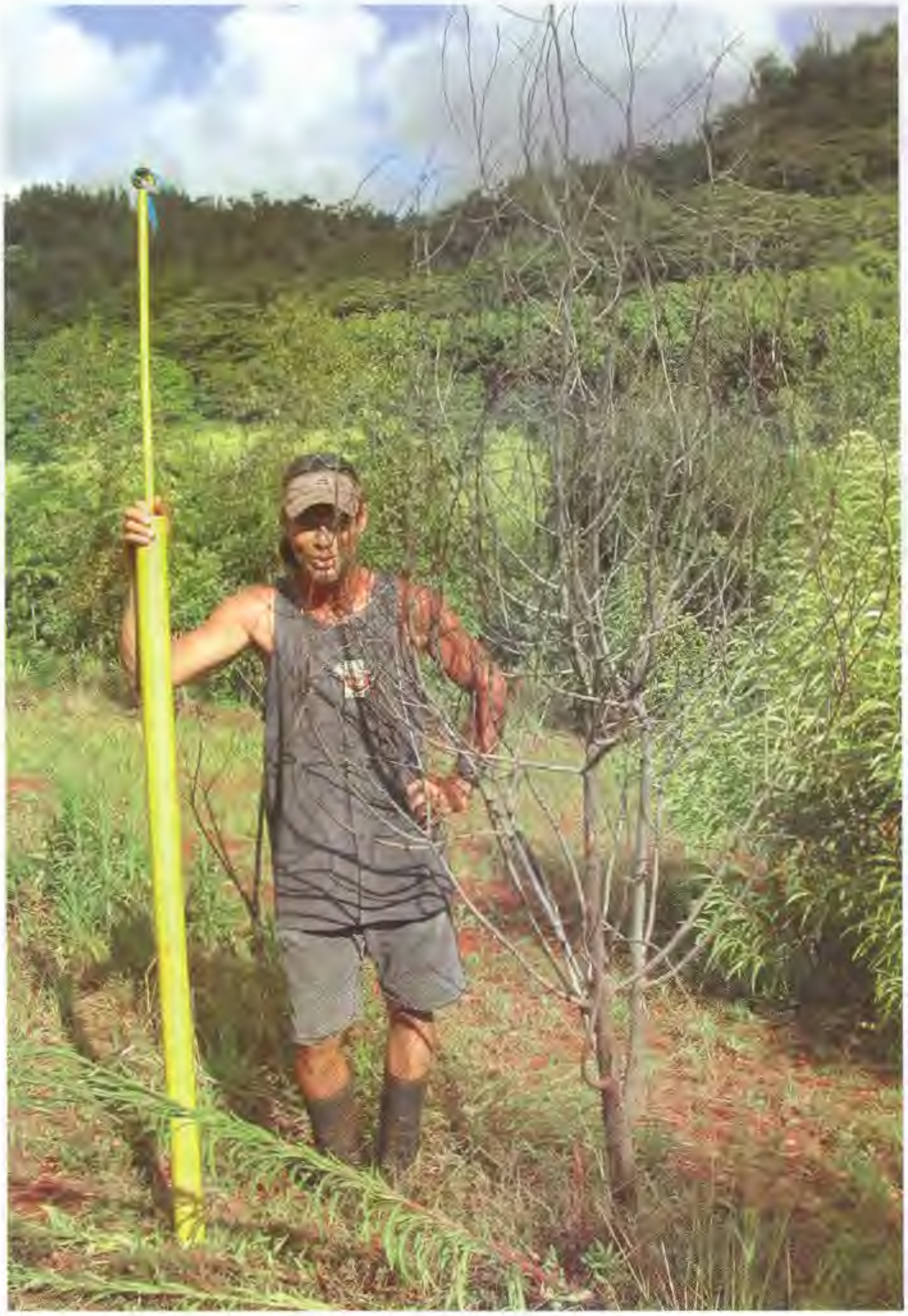

Figure 2. Wilting of *Acacia koa* sapling in Hawai'i.
Photo by NS Dudley

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