Investigating koa wilt in Hawai‘i
examining Acacia koa seeds and seedpods for Fusarium species

Robert L James, Nick S Dudley, and Aileen Yeh

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.


KEY WORDS
koa, wilt disease, seed infection, epidemiology

NOMENCLATURE
(fungi) Farr and others (1989);
(plants) USDA NRCS (2006)
A
cacia koa A. Gray (Fabaceae) is an
important tree species in Hawai‘i
from both an ecological and eco-
nomic 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 puta-
tively 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 O‘ahu,
Kaua‘i, Maui, and Hawai‘i (Big Island).
Traces of all ages, from seedlings to over-
mature, are affected. Damage is particu-
larly 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 colo-
nization 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 lev-
els 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 abun-
dance, 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 dis-
playing wilt/dieback disease symptoms.

Several species were isolated from
diseased trees on several sites on several
Islands (Tables 3 and 4). Similar Fusarium species also readily colonized
seedpods at the sampled sites (Table 5).
Several Fusarium spp., such as F. semi-
tectum, F. subglutinans (Wollenw. &
Reinking) P.E. Nelson, T.A. Toussoun &
Marasas, F. solani (Mart.) Sac., and F.
avenaceum, were isolated most frequently.
Most other species were isolated at low
levels, including F. oxysporum, the puta-
tive cause of koa wilt/dieback disease.
All of the fungi listed were contaminants
located externally on seed coats or colo-
ized 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.²

<table>
<thead>
<tr>
<th>Seedlot</th>
<th>Island location</th>
<th>Percentage <em>Fusarium</em>[^y]</th>
<th>Percentage clean[^x]</th>
<th>Other fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPR-5</td>
<td>O‘ahu</td>
<td>1.2</td>
<td>93.8</td>
<td>None</td>
</tr>
<tr>
<td>OPR-8</td>
<td>O‘ahu</td>
<td>0</td>
<td>100.0</td>
<td>None</td>
</tr>
<tr>
<td>Kahana-c</td>
<td>O‘ahu</td>
<td>0</td>
<td>91.3</td>
<td>Penicillium</td>
</tr>
<tr>
<td>Anahola-2</td>
<td>Kaua‘i</td>
<td>0</td>
<td>100.0</td>
<td>None</td>
</tr>
<tr>
<td>F45P-2</td>
<td>Maui</td>
<td>0</td>
<td>100.0</td>
<td>None</td>
</tr>
<tr>
<td>Kapa-6</td>
<td>Big Island</td>
<td>0</td>
<td>98.8</td>
<td>Penicillium</td>
</tr>
<tr>
<td>Kapa-5</td>
<td>Big Island</td>
<td>7.5</td>
<td>86.3</td>
<td>Penicillium Aspergillus Pestalotia</td>
</tr>
<tr>
<td>93-313-9</td>
<td>Big Island</td>
<td>1.3</td>
<td>97.5</td>
<td>Penicillium</td>
</tr>
<tr>
<td>All lots</td>
<td></td>
<td>1.25</td>
<td>95.9</td>
<td>——</td>
</tr>
</tbody>
</table>

[^y]: All isolates were *F. avenaceum*.
[^x]: Percentage of seeds without any fungi detected on seed coats.

² 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).
Contamination of Acacia koa stored seeds with superficial fungal mycelium with Fusarium species.\(^2\)

<table>
<thead>
<tr>
<th>Fusarium species</th>
<th>Percentage of sampled seeds colonized (^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>semitectum</td>
<td>55.4</td>
</tr>
<tr>
<td>sambucinum</td>
<td>12.0</td>
</tr>
<tr>
<td>solani</td>
<td>7.2</td>
</tr>
<tr>
<td>acuminatum</td>
<td>2.4</td>
</tr>
<tr>
<td>subglutinans</td>
<td>1.2</td>
</tr>
<tr>
<td>All Fusarium</td>
<td>78.3</td>
</tr>
</tbody>
</table>

\(^2\) Total number of seeds sampled = 83.

Fusarium species frequently 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

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

<table>
<thead>
<tr>
<th>Fusarium species</th>
<th>O‘ahu</th>
<th>Volcano National Park</th>
<th>Miscellaneous Kaua‘i</th>
<th>Sample location</th>
<th>Hamakua Research Station—Big Island</th>
<th>Miscellaneous Big Island</th>
<th>Kaua‘i</th>
<th>Opaeula Ridge—O‘ahu</th>
<th>All samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>acuminatum</td>
<td>5.0</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>avenaceum</td>
<td>12.5</td>
<td>17.0</td>
<td>3.9</td>
<td>4.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>equiseti</td>
<td>3.0</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>graminearum</td>
<td>13.0</td>
<td>2.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lateritium</td>
<td>12.6</td>
<td>0.5</td>
<td>4.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oxysporum</td>
<td>11.0</td>
<td>3.9</td>
<td>2.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>poae</td>
<td>18.8</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>proliferatum</td>
<td>12.0</td>
<td>1.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sambucinum</td>
<td>1.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>semitectum</td>
<td>2.2</td>
<td>89.7</td>
<td>7.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>solani</td>
<td>2.2</td>
<td>13.5</td>
<td>43.3</td>
<td>16.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sporotrichioides</td>
<td>10.0</td>
<td>3.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>subglutinans</td>
<td>37.8</td>
<td>40.9</td>
<td>19.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

\(^2\) 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.

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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 *F. solani*. We have frequently isolated *F. solani* from diseased trees, as have Daehler and Dudley (2002), particularly within the interior root and stem wood. *Fusarium 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 *Fusarium* species, *F. semitectum* and *F. subglutinans*, were consistently found at several locations on both seeds and seedpods. *Fusarium semitectum* 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 (Shahda 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-

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**Figure 1.** Size and morphological variation of different seedlots of *Acacia koa* seeds.
Photo by NS Dudley
ber 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 1985), 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). 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 *Hawai‘i*, 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 based on the basis of genetic analysis (O’Donnell 2005). It has been described only once before, associated with mango malformation in South Africa. *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 *Hawai‘i*.
### TABLE 5

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

<table>
<thead>
<tr>
<th><em>Fusarium</em> species</th>
<th>Waimano O‘ahu</th>
<th>Volcano National Park</th>
<th>Miscellaneous Kaua‘i</th>
<th>Sample location</th>
<th>Hamakua Research Station—Big Island</th>
<th>Miscellaneous Big Island</th>
<th>Miscellaneous Kaua‘i</th>
<th>Opauela Ridge Big Island</th>
<th>All samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>acuminatum</td>
<td>8.0</td>
<td></td>
<td>0.9</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>avenaceum</td>
<td>47.3</td>
<td>8.0</td>
<td>75.0</td>
<td>13.9</td>
<td>19.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>graminearum</td>
<td>6.0</td>
<td>9.3</td>
<td>0.9</td>
<td>3.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lateritium</td>
<td></td>
<td>26.0</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oxysporum</td>
<td></td>
<td>1.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>proliferatum</td>
<td></td>
<td>1.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sambucinum</td>
<td>0.9</td>
<td>11.0</td>
<td>1.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>semitectum</td>
<td>1.9</td>
<td>98.0</td>
<td>3.7</td>
<td>34.3</td>
<td>17.0</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>solani</td>
<td>1.9</td>
<td>3.0</td>
<td>13.9</td>
<td>36.1</td>
<td>9.0</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>sporotrichioides</td>
<td>0.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>subglutinans</td>
<td>47.2</td>
<td>3.0</td>
<td>20.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All <em>Fusarium</em></td>
<td>52.8</td>
<td>49.1</td>
<td>100.0</td>
<td>86.0</td>
<td>80.0</td>
<td>72.2</td>
<td>71.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Number of seedpod pieces sampled: 214, 110, 100, 100, 215, 108, 847

<sup>2</sup> 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. subglutinans* Britz, Marasas & Wingfield. All species are Hyphomycetes; some have teleomorphs in the genera *Gibberella* and *Nectria*.

### REFERENCES


Figure 2. Wilting of *Acacia koa* sapling in Hawai‘i.

Photo by NS Dudley


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