

Impact of the black twig borer, an introduced insect pest, on *Acacia koa* in the Hawaiian Islands

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Abstract—The Asian black twig borer (*Xylosandrus compactus* Eichhoff 1875) became naturalized in the Hawaiian Islands in the 1960s. It attacks economically important plants (e.g. coffee) as well as endangered endemic trees. This study examined the impact of the black twig borer on koa (*Acacia koa*), an economically important native tree that is harvested for its fine wood. The black twig borer tunnels into healthy branches, inoculating the wood with a fungus, *Fusarium solani*. We tested whether *F. solani* is pathogenic to koa by inoculating seedlings and saplings with the fungus. We found that *F. solani* was not pathogenic to koa. Nevertheless, in both natural and plantation settings the beetle caused significant mortality in koa seedlings and small saplings. Mortality was associated with boring into the stem, which often caused the main stem to break. The beetles also attacked live twigs on larger saplings and trees, causing flagging, but this damage was not correlated with tree mortality and did not appear to affect koa growth rates. The main source of mortality among older saplings was infection by the vascular wilt fungus, *F. oxysporum*. This fungus did not appear to be spread by the black twig borer. The main economic and ecological impact of the black twig borer on koa is in the seedling and small sapling stages. In a plantation setting, these impacts could be reduced by outplanting larger saplings and/or genetic selection for resistance. We found significant genetic variation among koa maternal families in rates of black twig borer attack.

Introduction

Many introduced insects have become pests of agriculture in the Pacific (Beardsley 1979, Schreiner 1991) but some of these alien insects also attack native plants in natural forests (Swezey 1954). One such example is the black twig borer, *Xylosandrus compactus* Eichhoff 1875 (Coleoptera: Scolytidae). The black twig borer is probably native to Southeast Asia. In Hawai'i, specimens were

first discovered in 1931 in elderberry imported from Singapore (Samuelson 1981), but the black twig borer was not reported as naturalized until around 1960 (Marsden 1979). Since then, it has become a serious pest of economically important plants including, *Eucalyptus* spp. avocado, coffee, citrus and *Acacia koa* (Marsden 1979). The black twig borer has also been introduced to the southeastern United States where it causes economic damage to avocado pear trees and other forest trees (Ngoan et al. 1976). The black twig borer is a serious pest because it typically infests young, live wood, even on apparently healthy trees (Nelson & Davis 1972) and saplings (Daehler, personal observation). In Hawai'i, at least 108 tree and shrub species belonging to 44 plant families are attacked by the black twig borer (Hara & Beardsley 1979). Large trees and shrubs have reportedly been killed by twig borer attack (Nelson & Davis 1972).

The black twig borer is a type of ambrosia beetle. Ambrosia beetles are well known as pests of trees and cut timber (Beal & Massey 1945, Orbay et al. 1994). These beetles bore galleries into sapwood and heartwood (Beal & Massey 1945), inoculating the galleries with an ambrosia fungus, which they usually carry in specialized pouch-like structures (mycangia). In a successful nest, the ambrosia fungus grows to line the gallery, stimulating maturation of the female's ovaries (Norris 1979), and later providing an essential food source for the brood (Beaver 1989). Thus, most ambrosia beetles cannot survive or reproduce without the successful growth of an ambrosia fungus (Beal & Massey 1945, Beaver 1989). Several ambrosia beetles, including the black twig borer carry *Fusarium solani* (Hyphomycetes) as their primary ambrosia fungus (Nord 1972, Norris 1979, Hara & Beardsley 1979). *Fusarium solani* is a well-known plant pathogen that can cause cankers, root rot, or rapid wilt syndrome, depending on the strain of *F. solani* involved (Booth 1971). Thus, there are two distinct mechanisms through which the black twig borer can potentially harm trees: direct physical damage and inoculation of the trees with a fungal pathogen.

Damage by the black twig borer to koa (*Acacia koa* Gray (Fabaceae), a tree endemic to the Hawaiian Islands, is of particular concern because of the rapidly growing koa wood industry in the Hawaiian Islands. Koa wood is prized for furniture, cabinets, floors, musical instruments, and other high-value products. The koa industry was estimated at \$29 million in 1993, with harvests only from unmanaged forests (Yanagida et al. 1993) and this value has been predicted to reach \$100 million per year in the next decade (Wilson 1996). As a fast-growing native species with high economic value, much research has recently been devoted to methods of koa propagation (Nagai 1996). Koa grows naturally at elevations ranging from 100 - 2400 m (Whitesell 1990), and there is particular interest in transforming abandoned sugar cane fields (altitude 100-500 m) into high-value koa plantations (Dudley 1996). Initial observations of experimental plantings seemed to suggest that koa often suffers from heavy damage by the black twig borer, although this damage is restricted to elevations below about 900 m (C. Daehler, personal observation). Cooler temperatures at higher elevations may limit the success of the black twig borer, which is native to tropical areas.

Unpublished studies by E. Trujillo (Department of Plant Pathology, University of Hawai'i) found that *F. solani* isolated from the mycangia of the black twig borer was strongly pathogenic in *Annona* spp. (soursop and custard apple) (Annonaceae), but detailed studies of the pathogenicity of *F. solani* in koa or the effects of physical damage by the beetle have not been conducted.

In this study we assessed damage by the black twig borer on nearly 3000 koa saplings in order to quantify the relationship between attack by the black twig borer and koa growth and survival. We also made experimental inoculations of koa with *F. solani* taken from the mycangia of the black twig borer to assess the pathogenicity of the ambrosia fungus being spread by the black twig borer. Because the black twig borer is abundant in many areas that could be utilized for koa plantations, an understanding of the relationship between the black twig borer, *Fusarium solani*, and koa damage is essential for development of the koa industry.

Methods

SEED SOURCES

Seeds of *Acacia koa* used for this study were collected from the islands of O'ahu, Maui, Kaua'i and Hawai'i as part of a larger effort to collect and store koa germplasm (Sun 1996). Each collection used in this study was made from a single tree. Trees were usually at least 100 meters apart and from widely scattered sites ranging in elevation from 100 to 1600 m. In a recent taxonomic treatment, two highly localized Hawaiian species, *Acacia kauaiensis* Hillebr. and *Acacia koaia* Hillebr., were lumped with *A. koa* sensu stricto and considered as a single species (Wagner et al. 1990); however, for this study, we used only seeds collected from *A. koa* sensu stricto (Wagner et al. 1990). Based on protandry and low seed-set following pollinator exclusion (Lanner 1965) as well as failure of seeds to develop following controlled self-pollinations (Sun & Dudley, unpublished data), the mating system of koa has been considered to be primarily outcrossing. Therefore, the seeds collected from individual trees are likely to be half-sib families.

COMMON GARDEN PLOTS

Common garden plots were established on the island of O'ahu in 1996, 1997 and 1999 at the Hawai'i Agriculture Research Center's Maunawili breeding station on the windward side of O'ahu. This site is at an elevation of 180 m and receives ca. 100-350 cm of rainfall annually. The average temperature at Maunawili is 23°C, while the soil order is ultisol (Lolekea series), with a pH of ca. 5.0. Prior to planting, seeds were nicked at the distal end with nail clippers to break dormancy. Nicked seeds were planted into 5-inch dibble tubes containing a mixture of Perlite (Black Magic Products, Elk Grove, CA) and peat moss (Sphagnum) (1:3), supplemented with 100 g/m³ Osmocote 14-14-14 fertilizer (Scotts-Sierra, Marysville, OH). This mixture was inoculated with *Rhizobia* (group C) isolated from wild *A. koa* (Niftal, 1000 Holomua Rd, Paia, Maui, Hawai'i). After four to six weeks in a greenhouse at Waimanalo, O'ahu (or

Maunawili for the 1999 planting), seedlings were transferred to outdoor benches in full sun and maintained for eight weeks prior to transplanting into the field plots. During this time, plants were saturated once per week with a solution (1.25 g/L) of Peters Professional Foliar Feed 20-20-20 (Scotts-Sierra, Marysville, OH). At the time of field planting, seedlings were 20-50 cm tall.

Prior to planting, field sites were disked and sprayed with Roundup (Monsanto, St. Louis, MO) to control weeds. A randomized block design was employed in each year. The 1996 and 1997 plantings consisted of two blocks, with each block containing 48 maternal families (1996) or 56 maternal families (1997). Within each block, each maternal family was represented by 16 saplings, planted in a 4x4 arrangement. The 1999 planting involved four blocks of 34 maternal families. Within the blocks, each maternal family was represented by 6 saplings, planted in a linear arrangement. Spacing between saplings in all years was 1 x 1.5 m. During the first three months, drip irrigation was used to increase transplant survival. Weed control was performed ca. every three months using the herbicide Fusillade (Zeneca Agro, Wilmington, DE) and/or a mechanical weed-whacker.

ASSESSING BLACK TWIG BORER DAMAGE, KOA GROWTH AND KOA SURVIVAL

All plants in the 1996, 1997 and 1999 plots were surveyed for growth, overall health, survival, and black twig borer damage every 2 to 6 months between November 1997 and December 2000. Growth was assessed by measuring sapling height. Overall health was scored qualitatively (good, poor, very poor, or dead). Poor health was defined as having 25-50% of branches or phylodes dying or dead. A rating of very poor meant >50% of branches or phylodes dying or dead. Damage specifically due to the black twig borer was measured in several ways. For the surveys conducted 4 to 6 months after planting, all black twig borer holes were counted on each plant, and the total length of damaged (senescent) branches was measured. In later surveys of larger saplings, twig borer attack rates were estimated by counting the number of holes encountered after searching each tree for 20 seconds, while damage was estimated by counting the number of dead, flagged (broken) branch tips on the entire tree. To determine whether mortality was spatially clumped, we employed a join-counts analysis using Rook's definition of contiguity in the 1999 plot (Sawada 1999).

In addition to monitoring the planted koa saplings, 72 naturally recruiting koa seedlings were monitored in a forest in Manoa Valley, O'ahu (altitude 120 m) for period of 1 month in October 1996. These observations allowed assessment of twig borer damage on naturally occurring seedlings (3-30 cm tall) growing within natural forest vegetation.

ISOLATING FUNGI FROM THE BLACK TWIG BORER

Live adult specimens of the black twig borer were collected from within the koa plots at Maunawili as well as from within natural forest in Manoa Valley, O'ahu. Beetles were either removed from branch surfaces on which they were crawling, removed from galleries, or collected from ethanol-baited traps

hung from trees (trap design modified from Lindgren (1983)). In the laboratory, the live beetles were surface sterilized by submerging in 0.06% sodium hypochloride for 5 minutes. The beetles were then rinsed in three successive sterile water baths and blotted dry on sterile filter paper. Using a dissecting scope and sterile dissecting needle, the intersegmental region between the pro- and mesonotum of the beetle (area containing the mycangium) was separated from the rest of the body and transferred to sterile water agar. For some beetles, the entire body was placed on water agar. After 8 days on water agar at 23°C, fungal isolates were transferred to potato dextrose agar (PDA) and grown for approximately 14 days. The fungi were identified using FusKey, an interactive key for *Fusarium* species developed by Dr. Keith Seifert (published on the Internet at <http://res.agr.ca/brd/fusarium/home1.html>).

ISOLATING FUNGI FROM DYING TREES

A syndrome was frequently observed in the field plots wherein koa trees appeared wilted; the wilted phyllodes would drop, and the entire tree would die, sometime in a matter of days. To test whether *F. solani* could be responsible for this syndrome, we cultured fungi from the wood and roots of the dying trees. We also sampled wood and roots from healthy-looking trees as a control. Branches and roots were cut from the trees, placed individually in sterile plastic bags, and brought to the lab for culturing within 12 hours. Wood and roots were split open using a sterile scalpel, and samples of internal plant tissues were placed on water agar for 8 days. Fungi were transferred to PDA and identified after about 14 days using FusKey.

INOCULATING KOA WITH FUNGAL ISOLATES

To experimentally test whether *F. solani* isolated from the black twig borer was pathogenic to koa, six-week old seedlings were inoculated with *F. solani* using a stem inoculation method that was meant to simulate inoculation by the boring beetles. The seedlings were grown in 260 mL plastic cups containing a mixture of Perlite (Black Magic Products, Elk Grove, CA) and peat moss (Sphagnum) (1:3) supplemented with 100 g/m³ Osmocote 14-14-14 slow release fertilizer (Scotts Company, Marysville, Ohio). An incision 5 mm long and about 1.5 mm deep was created at the base of each seedling stem using a sterile dissecting needle. A visible mass of *F. solani* hyphae and spores (taken from an actively growing 7-day old PDA culture) was then inserted into the wound using a second dissecting probe. Control seedlings were wounded in the same manner but not inoculated. Some seedlings were also inoculated with isolates obtained from the dying trees for comparison with isolates taken from the beetles. In one experiment, soil inoculation was used to determine if *Fusarium* isolated from dying trees could infect koa via the roots. Following Sands et al (1997), millet was sterilized by autoclaving and inoculated with *Fusarium* isolated from dying koa. When the culture was 14 days old, the millet was mixed with potting medium (1:5 v/v millet to potting medium), and seedlings were transplanted into the mixture.

Controls consisted of potting medium mixed with sterile millet. All seedlings were placed in a 1 m x 2 m vented plexi-glass greenhouse (natural sunlight, located to the University of Hawai'i at Manoa) and watered daily as needed to keep the soil moist. Seedlings were monitored for 4 to 16 weeks (depending on the experiment) and scored for mortality and/or disease symptoms.

In addition to seedling inoculations of greenhouse-grown plants, 4 healthy-looking koa saplings (2-3 meters tall) in the 1996 field plot were inoculated with *F. solani* isolates from the black twig borer to test for pathogenicity. Four branches, approximately 1 cm in diameter each, were selected on each tree, and an incision approximately 1 cm long and 3 mm deep was made on each branch using an ethanol-sterilized pocket knife. Each tree was inoculated with three different *F. solani* isolates, one per wounded branch. Using a dissecting needle, the wounds were filled with a visible mass of hyphae and spores taken from a PDA plate. The fourth wounded branch was not inoculated and served as a control. The wounded branches were monitored for six weeks.

Results

FIELD SURVEY OF NATURAL KOA SEEDLINGS

Among the 72 naturally recruiting koa seedlings monitored for 1 month, 17 (24%) were attacked by the black twig borer. Some of the seedlings monitored were probably too small to attract the black twig borer because they were less than



Figure 1. Young koa sapling with damage from the black twig borer. Main trunk has broken at the site of boring.

0.3 cm in diameter (Hara & Beardsley 1979). Considering only the koa seedlings > 25 cm tall (usually with main stems at least 0.4 cm in diameter at the base), the attack rate rose to 77%. Among those plants attacked, 76% died. Koa death was usually associated with breaking of the main stem after tunneling by the black twig borer (Fig. 1).

SURVEY OF PLANTED KOA – SMALL SAPLINGS

Ten weeks after planting, saplings in the 1997 planting averaged 70 cm tall (standard deviation, SD = 28). From among 1263 saplings surveyed at that time, 12% had been damaged by the black twig borer. Among the attacked saplings, 35% had died. In contrast, the mortality rate among saplings that were not attacked by the black twig borer was significantly lower (18.9%, chi-squared = 17.3, $P < 0.0001$). Damage and/or death to the attacked plants was primarily due to breaking of the main stem at the site of boring. Less often, wilting was observed distal to the point of black twig borer attack. In contrast, the koa saplings that died without twig borer attack appeared to have died very soon after transplanting, probably due to drought stress associated with transplant shock or - accidental misting with herbicide during weed control efforts in the plot. Surveys from the 1999 planting revealed a similar trend. The first survey took place 8

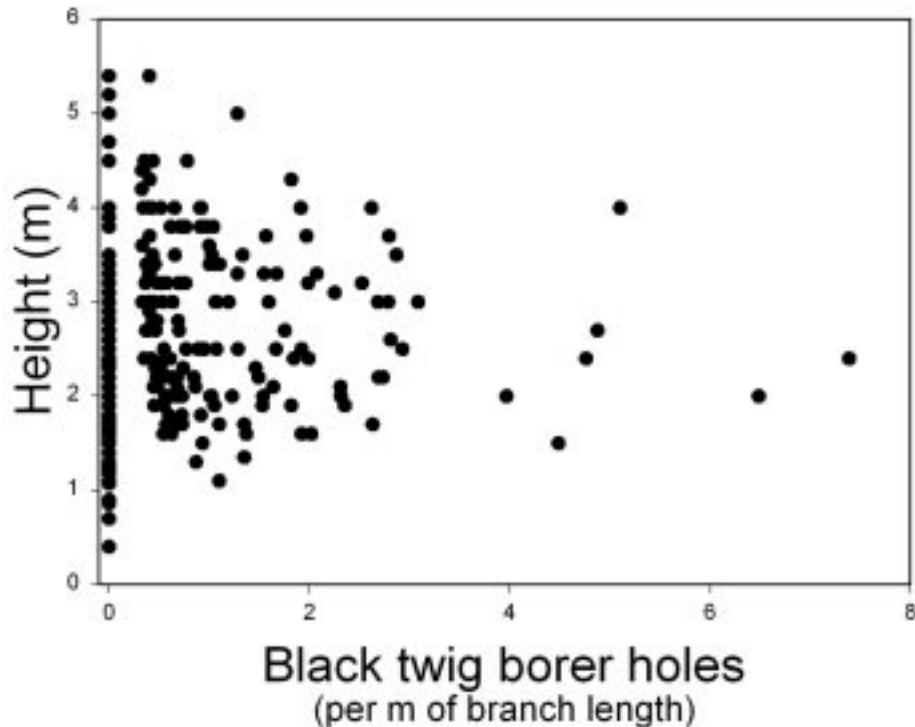


Figure 2. Height versus number of black twig borer holes (per meter of branch length) in the 1996 plot. Higher attack rates did not result in smaller trees.

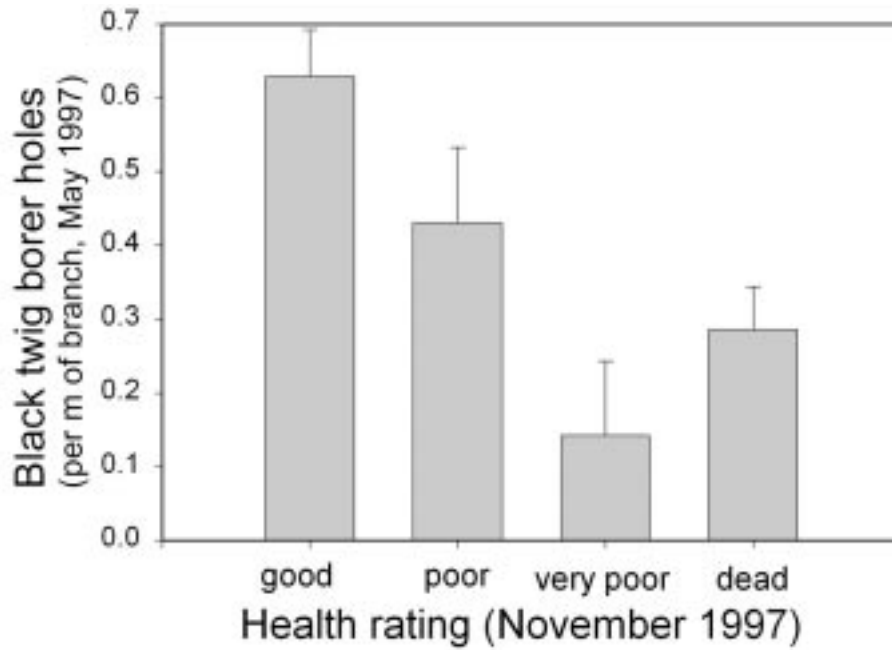


Figure 3. Health status versus number of black twig borer holes (per meter of branch length) in the 1996 plot.

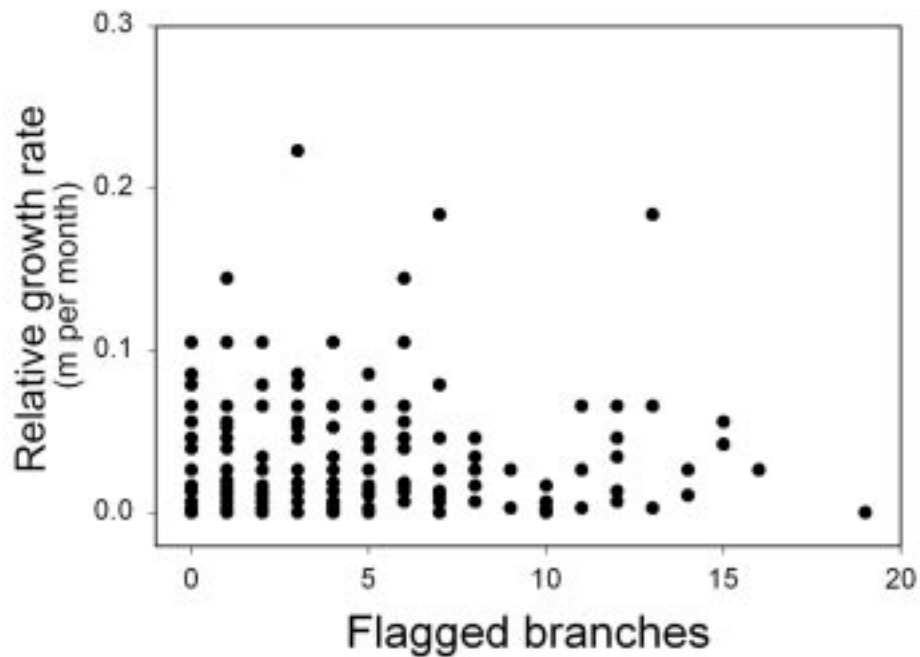


Figure 4. Relative growth rate versus black twig borer damage in the 1997 plot.

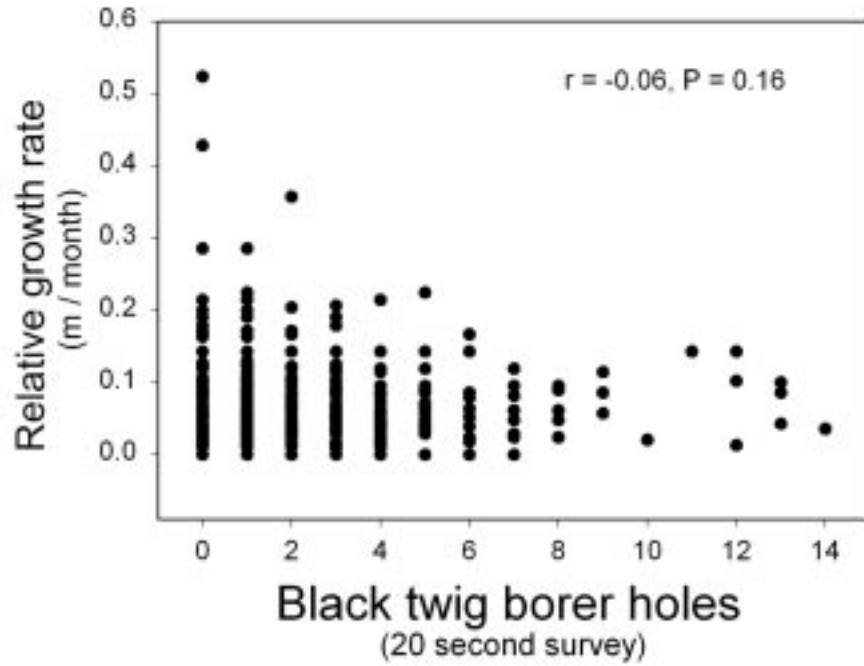


Figure 5. Relative growth rate versus black twig borer attack rate in 1999 plot.

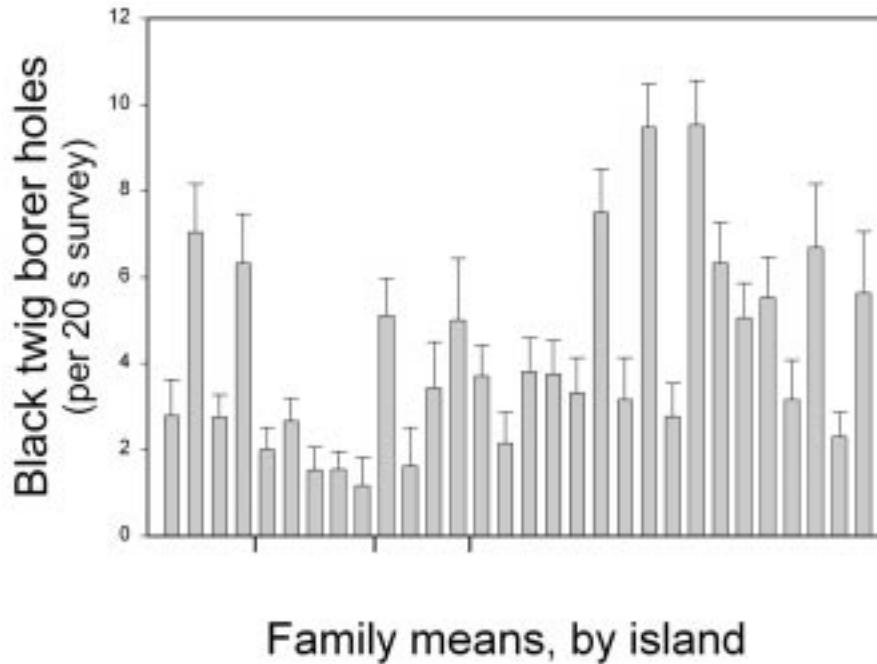


Figure 6. Black twig borer attack rate versus koa maternal family in the 1999 plot.

weeks after planting, when saplings averaged 54 cm in height (SD = 15). From among 768 saplings surveyed, 8.4% were damaged by the black twig borer. Among the attacked saplings, 37% had a health rating of very poor and 25% had died. The mortality rate among saplings not attacked by the black twig borer was significantly lower (4%, chi-squared = 37.6, $P < 0.0001$).

SURVEY OF PLANTED KOA – LARGER SAPLINGS

As the surviving saplings grew, the role of the black twig borer in causing mortality declined. For example, by October 2000, the surviving saplings in the 1999 planting averaged 310 cm (SD= 110). The mortality rate of these large saplings between March 2000 and October 2000 was high but did not differ between saplings with and without black twig borer damage (17.6% versus 16.1% mortality, respectively, chi-squared = 0.2, $P = 0.7$). Furthermore, among larger saplings in all three planting years, there was no relationship between the degree of black twig borer damage and the health, height, or relative growth rate of koa. In the 1996 koa planting, which was first surveyed when saplings averaged 260 cm tall (November 1997) sapling height was not related to the number of black twig borer holes per branch length (Fig. 2, linear regression, $P = 0.17$). Similarly, koa health status in November 1997 was not negatively impacted by the number of black twig borer holes in May 1997 (Fig. 3). In fact, there was a trend towards saplings with more black twig borer holes in May 1997 being healthier in November 1997 (Kruskal-Wallis test, $P = 0.055$, Fig. 3).

The same trend was observed among older saplings in the 1997 planting. By December 1999, the koa trees averaged 320 cm (SD = 120) in the 1997 plot, and there was no correlation between amount of twig borer damage observed in December 1999 (as measured flagging on branches) and relative growth rate of the trees between December 1999 and November 2000 (Fig. 4). In the 1999 plot, relative growth rate of the trees between March and October 2000 was uncorrelated with the number of black twig borer holes observed in March 2000 (Fig. 5). In all three plots, some maternal koa families suffered significantly less attack by the black twig borer than others (e.g., Fig. 6 for the 1999 plot, ANOVA, $F_{29,88} = 9.0$, $P < 0.001$) but these differences were not related to the maternal plant's island of origin (ANOVA, $F_{4,16} = 1.46$ $P = 0.3$). Statistical analysis of the spatial pattern of mortality within the 1999 plot indicated that mortality following the first survey (when saplings were small) was randomly distributed within the plot, but after the second survey (larger saplings) the dying koa trees were clustered together (Join-counts analysis, $Z = 3.69$, $P < 0.05$).

FUNGAL ISOLATES FROM THE BLACK TWIG BORER, GALLERIES AND DYING KOA SAPLINGS

Fungi were cultured from 36 live black twig borers in 1998, and in all cases *F. solani* was discovered. An additional 110 beetles were surveyed in December 2000. For these beetles, we also attempted to isolate fungi from the abdomen and/or the entire beetle. Most of these beetles carried *F. solani*, although a second



Figure 7. Dying koa saplings from the 1999 plot. Culturing of wood yielded *F. oxysporum*.

ambrosia fungus, *Ambrosiella xylebori* Brader ex Arx & Hennebert (Hyphomycetes) was also isolated in some cases. When the wood surrounding black twig borer galleries was sampled, *F. solani* was always isolated (n = 18), but *F. solani* was rarely found in wood more than 10 cm from a gallery in the proximal end of the bored branch. *F. solani* infection was confined to the site of the gallery and the distal end of the bored branch.

In contrast to wood sampled from black twig borer galleries which yielded *F. solani*, wood cultured from undamaged branches of dying koa saplings (n = 9, Fig. 7) yielded *F. oxysporum*. In these unhealthy trees, *F. solani* was only found in the immediate vicinity of twig borer damage. Scraping away the outer tissue of the woody roots on these dying trees revealed pinkish stains that are symptomatic of *F. oxysporum* infection. Root samples cultured from these trees (n = 5) yielded *F. oxysporum*, not *F. solani*. Wood and root samples from five healthy-looking koa saplings did not yield *F. oxysporum*.

FUSARIUM INOCULATIONS

Stem inoculations of koa by *F. solani* isolated from the black twig borer did not increase koa mortality relative to uninoculated controls (Fig. 8), and the inoculated koa plants did not exhibit disease symptoms. A mortality rate of about 20% was observed in both inoculated and control plants in the first experiment, and

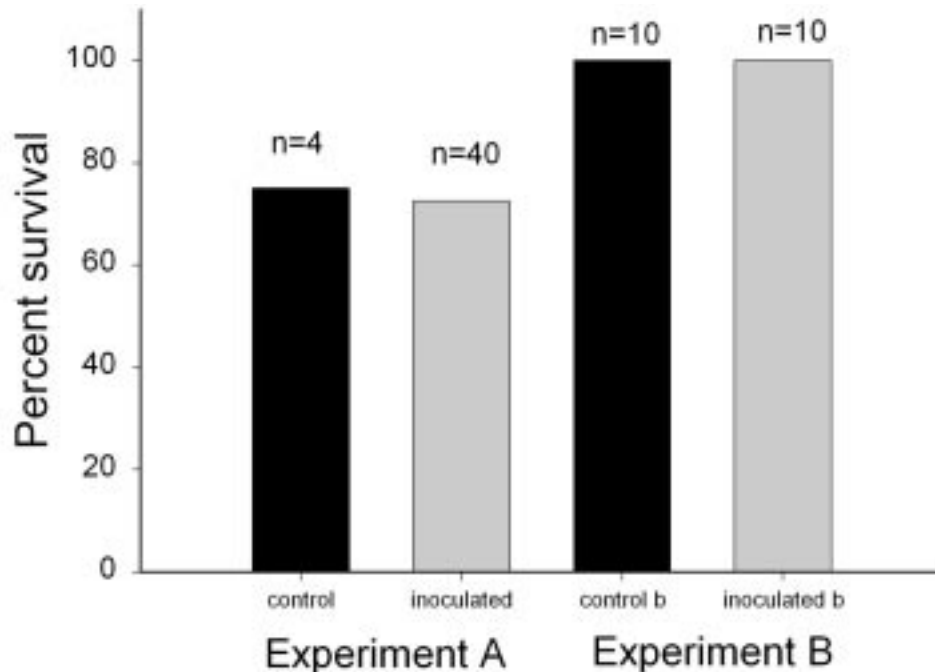


Figure 8. Stem inoculation of koa seedlings with *F. solani* isolated from the black twig borer. Experiment A was initiated in April 1998 and the results were scored 8 weeks later. Experiment B was initiated in January 1999 and the results were scored 16 weeks later.

this mortality was probably due to drought stress associated with a missed watering period. The four field saplings inoculated with *F. solani* did not exhibit disease symptoms. A localized wound response was observed on each inoculated branch. Most wounds exuded a sticky sap, which was sometimes black in color. Three weeks after inoculation, tissue necrosis did not extend more than 1 cm away from each wound. Six weeks after inoculation, scarring was still obvious at the inoculation sites, but regrowth of the wood tissues had occurred, and the wound site appeared healthy with no signs of rotting or continued necrosis. The *F. solani*-inoculated wound response did not notably differ from the control (uninoculated) wound response.

Stem inoculations of koa seedlings with *F. oxysporum* resulted in higher seedling mortality, relative to uninoculated controls (Fig. 9). Typically, the *F. oxysporum*-inoculated seedlings dropped their leaves, and the stems became shriveled. These symptoms were similar to the rapid dropping of phyllodes by dying saplings in the field. Soil inoculation with *F. oxysporum* also led to increased mortality of koa seedlings relative to controls (Fig. 9). Field inoculations of koa with *F. oxysporum* were not carried out.

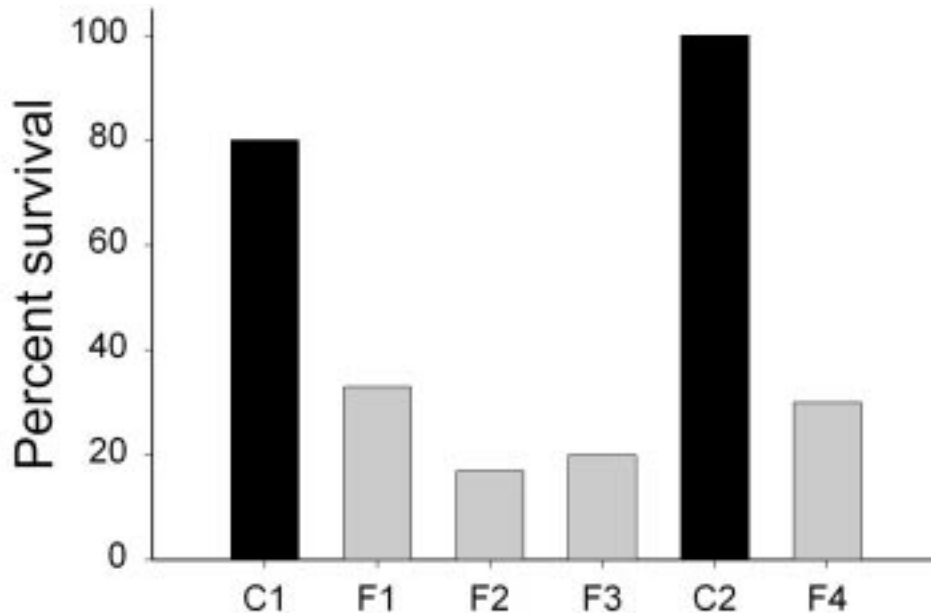


Figure 9. Inoculation of koa seedlings with *F. oxysporum* by stem inoculation (F1-F3 = three different isolates from dying koa, C 1 = control) or by root inoculation (F4 = a fourth isolate from dying koa, C2 = control). The stem inoculation experiment was initiated in June 1998 and scored after 8 weeks, while the root inoculation experiment was initiated in April 1999 and scored after 4 weeks.

Discussion

AMBROSIA FUNGUS-PLANT INTERACTIONS

Although there is a large literature on the fungal pathogens spread by bark beetles (Cates & Alexander 1982, see also bibliography by Wood 1984), the pathogenicity of ambrosia fungi spread by ambrosia beetles, such as the black twig borer, has been little studied. This is probably because most ambrosia beetle species attack dead or cut wood (Bright 1968). The black twig borer is unusual in its habit of attacking apparently healthy, green wood. Weber & McPherson (1985) reported that *F. solani* spread by *Xylosandrus germanus* Blandford (Coleoptera: Scolytidae) was associated with black walnut canker, but these cankers are usually localized at wound sites (Carson 1994). Similar cankers on tulip poplar trees in Ohio were associated with ambrosia beetle attack and *F. solani* infection (Anderson & Hubbard 1978). Ambrosia beetles attacking *Angsana* (*Pterocarpus indicus*) in Singapore were also found to carry *F. solani* as the ambrosia fungus, but seedling inoculations showed that *F. solani* was not pathogenic to *Angsana*; inoculated seedlings appeared just as healthy as uninoculated seedlings (Sanderson et al. 1996). We found that *F. solani* carried by the black twig borer was at most only weakly pathogenic to koa, resulting in a localized infection around the site of boring that sometimes spread to the distal end of the bored

branch. Since the black twig borer is limited to attacking small branches, the damage was not great. Observations on other plants attacked by the black twig borer near our field plots, including *Coffea arabica* L. (Rubiaceae), *Citharexylum caudatum* L. (Verbenaceae), and *Desmodium tortuosum* (Sw.) DC. (Fabaceae), indicated that necrosis was similarly limited to the distal portion of the branch containing the gallery; necrosis rarely extended more than 10 cm from the bore hole in the direction of the main trunk. Stem inoculations of koa seedlings and field inoculations of koa saplings with *F. solani* taken from the black twig borer failed to induce mortality; inoculated plants were as healthy as the uninoculated control plants. Failure of *F. solani* to spread to the distal end of experimentally inoculated branches suggests that extensive or prolonged damage due to active boring by the beetle is necessary for *F. solani* to spread distally on a branch. The second ambrosia fungus isolated from the black twig borer, *Ambrosiella xylebori* has not been reported as a pathogen of any plant, was never isolated from koa wood, and is unlikely to impact koa as a pathogen. The ambrosia fungi carried by the black twig borer do not appear harmful to large koa trees. Nevertheless, more than 100 plant species have been reported as hosts of the black twig borer in Hawai'i alone (Hara & Beardsley 1979), and we cannot exclude the possibility that *F. solani* spread by the black twig borer could systemically infect and kill other, more susceptible tree species.

PHYSICAL DAMAGE BY THE BLACK TWIG BORER

In contrast to the weak pathogenicity of the ambrosia fungus, physical damage due to boring often killed small saplings (< 1 m in height) when the bore hole was at the base of the main trunk. Boring weakened the trunk making it susceptible to breaking by wind. Sometimes, these bored saplings wilted and died without breaking. This wilting was probably due to physical damage to the xylem conduits, rather than the spread of *F. solani*, since wilting often occurred within hours of black twig borer attack. Physical damage by the black twig borer may be costly during the first few months of plantation establishment at low elevations (below about 900 m) where the black twig borer is abundant. Furthermore, given the observed high rate of black twig borer attack among koa seedlings in natural areas, the beetle can also be expected to limit recruitment of koa in natural forests at low elevations.

In larger saplings (1.5 to 4 m in height), black twig borer damage was not associated with koa mortality. On large trees, the black twig borer did kill some small branches or branch tips, but the effect on overall koa growth was not statistically detectable, even with our large sample size of over 1000 trees. Furthermore, saplings rated as healthy had more black twig borer holes than saplings with a poor or very poor health rating, underscoring the lack of relationship between black twig borer attack and poor health. We observed other wood boring beetles (e.g. *Xylosandrus crassiusculus* Motschulsky (Coleoptera: Scolytidae) and *Xyleborus* spp. (Coleoptera: Scolytidae)) on the trunks of larger koa saplings, and these species would seem to have greater potential to cause

harm to large koa, in comparison to the black twig borer, which restricts its boring to relatively small branches of about 15 mm diameter or less (Marsden 1979, Hara & Beardsley 1979).

MORTALITY IN LARGER SAPLINGS

Mortality in large koa saplings was not associated with black twig borer damage, but was instead associated with infection by *Fusarium oxysporum*. *F. oxysporum* is chiefly a soil saprophyte, but some strains are serious wilt pathogens of plants (Booth 1971). Gardner (1980) first described *F. oxysporum* f. sp. *koa* as a wilt pathogen of koa seedlings. The pathogen was observed in greenhouse-grown koa seedlings from the island of Hawai'i, but the extent of distribution of the pathogen on other islands was not investigated (Gardner 1980). A follow-up study was conducted by Anderson et al. (2002), wherein *F. oxysporum* was isolated from the roots and wood of dying koa trees within Hawai'i Volcanoes National Park (island of Hawai'i). These *F. oxysporum* isolates were pathogenic to koa seedlings in root inoculations, and are presumed to be *F. oxysporum* f. sp. *koa*, originally described by Gardner (Anderson et al. 2002). The dying koa sampled on the island of Hawai'i occurred at elevations between 1300 and 1650 m, and no signs of black twig borer damage were observed on these trees (Anderson et al. 2002). The pathogenic *F. oxysporum* we isolated from koa saplings on O'ahu is similar to that reported by Anderson et al. (2002), and although it may not be genetically identical, it can be referred to as *F. oxysporum* f. sp. *koa* based on its pathogenicity to koa (Gardner 1980).

IS *F. OXYSPORIUM* SPREAD BY THE BLACK TWIG BORER?

In studying the relationship between an ambrosia beetle (*Platypus parallelus* F. Coleoptera: Platypodidae) and dieback of Angsana trees in Singapore, Sanderson et al. (1996) reported that up to 40% of ambrosia beetles that were captured were carrying *F. oxysporum* propagules, and *F. oxysporum* was associated with Angsana dieback. They concluded that the ambrosia beetle was a major vector of *F. oxysporum* wilt, but secondary infections could also occur in neighboring trees via the soil. The situation appears different in the relationship between the black twig borer and koa. From among more than 100 black twig borers sampled, we never isolated *F. oxysporum*, only *F. solani*. Furthermore, if the black twig borer were an important vector of *F. oxysporum*, we would have expected saplings with a higher black twig borer attack rate to have had a higher probability of dying. This was not the case for saplings in any of our plots, except for the first sampling date when saplings were small and boring led to breaking of the main stem.

Some indication of how *F. oxysporum* spreads comes from the spatial analysis of mortality in the field plots. During the first survey, much mortality was caused by the black twig borer, but the spatial distribution of mortality was not clumped within the plot. In later surveys, the spatial distribution of mortality became statistically clumped. There were obvious patches of dead koa, and these

patches tended to grow over time. This pattern of spread is consistent with soil-born spread of the pathogen to neighboring trees via roots. We cannot eliminate the possibility that some spread to neighboring trees was by an insect, but some dying saplings were not attacked by the black twig borer, demonstrating a different mode of infection. Furthermore, as previously pointed out, if the black twig borer were an important vector, we would expect to see a relationship between number of black twig borer attacks and risk of mortality. We did not find such a relationship.

Assuming that soil is the main vector of *F. oxysporum*, and the black twig borer is unimportant, how did *F. oxysporum* arrive in the field plots? The plots had previously been used for agriculture, and did not contain koa for decades (if ever). Gardner (1980) reported that *F. oxysporum* was found inside koa seeds, resulting in seedling infection. This conclusion was based on the appearance of *F. oxysporum* f. sp. *koa* in greenhouse-grown seedlings even after the seed coat and potting medium had been sterilized. Therefore, one possible source of *F. oxysporum* in the field is the outplanted seedlings. After an infected seedling has been planted, it may take some time for the fungus to spread through the soil to reach neighboring plants, explaining the appearance of a clumped pattern of mortality only after the first survey. A second possible source of *F. oxysporum* in the plots is the larger, trunk-boring beetle species that were discovered only during later sampling periods in the larger saplings. Attacks by these larger wood-boring beetles on the older saplings could also help explain why the koa mortality rate remained high throughout the study (about 20% per 6 month period), even among well-established trees. Further studies of these trunk-boring beetles are warranted to determine whether they might be important in establishing new foci of *F. oxysporum* infection.

Conclusions

The black twig borer is an important source of mortality in small koa saplings (< 1 m tall). One strategy for limiting mortality due to the black twig borer would be to outplant larger saplings. An insecticide sprayed on the main trunk of small koa saplings could also discourage attack (Marden 1979), but is unlikely to be effective after the beetle has penetrated the wood. We found significant genetic variation in susceptibility to black twig borer attack in koa, so a long term strategy would be to select for resistant lines of koa.

Once the koa saplings exceeded 2 m in height, very little mortality could be attributed to the black twig borer. This finding is contrary to a previous report by Nelson & Davis (1972) in which vigorous trees of *Melaleuca quinquenervia* (Cav.) S.T. Blake (Myrtaceae), *Syncarpia glomulifera* (Sm.) Nied. (Myrtaceae), *Tristania conferta* R. Br. (Myrtaceae), and *Eucalyptus* spp. (Myrtaceae) were seemingly killed by the black twig borer. In that study, potentially pathogenic microorganisms were also isolated from the trees, and it is difficult to distinguish effects of the black twig borer from the microorganisms,

which may or may not have been introduced by the black twig borer. We identified the vascular wilt pathogen *F. oxysporum* as the main source of mortality among larger koa saplings, but we found no evidence that the black twig borer serves as an important vector of this pathogen. Additional studies are needed to identify the most common vector(s) of *F. oxysporum* so that losses of koa can be minimized. Environmental variables such as soil pH and moisture seems to affect koa's susceptibility to *F. oxysporum* (Anderson et al. 2002) but additional studies on optimal growing conditions, as well as genetic variation in susceptibility to *F. oxysporum* will be important for obtaining reliable koa wood yields in planted stands.

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