

Role of Fungal Diseases in Decline of Pacific Madrone

Abstract

There is concern over the health of Pacific madrone, a tree native to California, Oregon, Washington, and British Columbia. Declining trees have been reported in western Washington and British Columbia during the past 30 yr. The fungus *Natrassia mangiferae* causes cankers and shoot blight and is associated with declining madrones. *Fusicoccum aesculi* causes branch die-back but is a secondary pathogen that attacks stressed trees. We found both fungi on declining madrone trees. More canker fungi were isolated within the first cm from the canker margin, and occurred more often on large diameter wood. *Natrassia mangiferae* was found in 90% of samples from the margin of madrone cankers and is considered the primary pathogen. Trees that were heavily infected with canker fungi had less stored starch in the root burl. Starch content in the root burl of declining trees was significantly lower than in healthy trees. Pacific madrone decline has a similar pattern to other declines involving early successional species that establish after a disturbance, where mature trees are more severely affected. Fire was the major natural disturbance agent with madrone, but disease now appears to be replacing fire as the main disturbance agent responsible for killing aboveground plant parts. Unlike fire, disease decreases starch accumulation in the root burl, so that declining trees are less able to resprout after the aboveground portion of the tree is killed by disease.

Introduction

A decline in Pacific madrone (*Arbutus menziesii*) has been reported during the past 30 yr in the Pacific Northwest (Davison 1972, Bressette 1995), and there is great concern about the health of this species that is native to the western U.S. and British Columbia. The problem was first seen in urban areas, but declining trees are also found in natural forests. All ages and sizes of madrones are affected, with larger, older madrones having the most mortality. The major cause appears to be a canker disease caused by the fungus *Natrassia mangiferae* (Deuteromycota) (Davison 1972, Hunt et al. 1992, Bressette 1995) (Figure 1). The first cases of cankers and declining madrones in Washington State were reported after a hot summer in 1967 followed by an unusually cold winter in 1969 (Davison 1972).

Natrassia mangiferae may be an introduced fungus since it first appeared in the 1960s in Washington, and probably spread to madrone from Persian walnut in California, where it causes a wilt disease (Paxton and Wilson 1965). Low disease resistance in madrone, severe weather conditions, and the practice of fire suppression in the 20th century may have allowed this typically weak pathogen to become a serious problem on Pacific



Figure 1. Declining madrone trees and associated cankers. The large, callusing cankers are typical of *Natrassia mangiferae* infections.

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madrone. Greater weakening of trees through defoliation is caused by the opportunistic pathogen *Fusicoccum aesculi* (Deuteromycota).

A fungus similar to *Natrassia mangiferae* was first reported in the U.S. on Persian walnut in California in 1947 (Wilson 1947) and named *Exosporina fawcetti*, later renamed to *Hendersonula toruloidea*. *Hendersonula toruloidea* was later placed in its own genus, *Natrassia*, of which *N. mangiferae* is the type species (Sutton and Dyko 1983). The fungus was originally found on plum, apricot, and apple (Natrass 1933) and has been reported from many tropical and sub-tropical countries where it is a weak pathogen that colonizes injured wood (Paxton and Wilson 1965, English et al. 1974, Sutton and Dyko 1989). Heat injury to the bark is required in most cases. Hot, dry weather encourages the development of disease symptoms (Calavan and Wallace 1954, Davison 1972, Pandey et al. 1981, Jayasingh and Silva 1994, Msikita et al. 1997). *Natrassia mangiferae* is especially destructive on thin-barked trees that grow where summer heat is intense. In addition to being a plant pathogen, the fungus causes skin disease on humans and other animals (Sigler et al. 1997). In both plant and animal hosts, infection by *N. mangiferae* is not normally a problem unless the host is under some type of stress.

On Pacific madrone, *N. mangiferae* infects the phloem and vascular cambium and causes shoot blight. Infection courts are generally created by mechanical injury or sunscald. Shaded trunks of madrone trees develop a thick layer of bark that is thought to protect the tree from fungal infection (Bressette 1995). Heat-injured bark and wounds become infected with conidia of *N. mangiferae*. Cankers first appear as an area of purplish to black bark discoloration. The bark peels off revealing longitudinally cracked wood and dark masses of asexual fungal spores (Figure 2). Conidia (arthrospores) are present all year in the dead wood of the canker and are carried by the wind to infect new hosts (Hunt et al. 1992). Inner bark and sapwood in the area around the cankers is discolored brown in contrast to healthy tissue, which is white or pale green.

Fusicoccum aesculi (*Botryosphaeria dothidea* (Ascomycota), teleomorph) has also been associated with declining madrone trees, and a large proportion of mature trees in northern California are dying from cankers and branch dieback caused

by this fungus (McDonald and Tappeiner 1991). On Pacific madrone, *F. aesculi* causes branch dieback where the limbs and twigs appear burned (Adams and Kosta 1993, Adams et al. 1999). Tiny black pycnidia are visible on the leaves and twigs. On many hosts this fungus can be present in the wood without causing disease, growing saprophytically on dead tissue or remaining latent until the water content of the wood falls below a critical level. Symptoms develop when the host becomes drought-stressed (Houston 1984, Pusey 1989, Boyer 1995).

When madrone branches and terminal buds are killed by fungi, they are unable to produce more foliage. The tree resprouts from dormant buds on the stem or root burl and grows new shoots to increase the amount of foliage in a manner similar to that occurring after a fire (USDA Forest Service 2002). These new shoots are often attacked and killed by *N. mangiferae*.

Aging madrone trees develop a large root burl with a spreading root system, which allows the trees to resprout when damaged by fire or disease. Dormant vegetative buds used in resprouting are located just above the root collar and grow rapidly when released. Trees that have lost much of their crowns from canker fungi and dieback will resprout if these vegetative buds are not damaged by cankers. Below the root collar madrones have a root burl, or lignotuber, which contains stored starch. Resprouting species allocate more carbon to storage in the root burl than do species that reproduce primarily by seed (Bell and Ojeda, 1999). Trees 50-60 yr old have a well-developed root burl with deep, spreading lateral roots (McDonald and Tappeiner 1991). These large trees are susceptible to heart rot, which makes them desirable for cavity-nesting birds (Raphael 1987).

Dominant and codominant individuals of early successional tree species that establish after a disturbance tend to show decline symptoms before younger members of the stand. This phenomenon is called cohort senescence (Gerrish 1993, Mueller-Dombois 1993) and is a natural part of the species life cycle. Large, old madrones are more susceptible to decline because madrone is an early successional species adapted to frequent fires (McDonald and Tappeiner 1991, USDA Forest Service 2002). Madrones have a growth rather than a defense strategy, which allows young plant parts, such as resprouts or seedlings, to be



Figure 2.

competitive in early (Loehle 1987). When mortality and senescence increased ratio of photosynthesis to leaves. Rapid response as a defense and the attack by pathogens. Defoliated by pathogen

and Tappeiner 1991). *Arctia* causes branch die-backs (Lambert et al. 1999). Tiny cankers appear on the leaves and twigs. *Arctia* can be present in the canopy, growing saprophytically, remaining latent until the tree falls below a critical point when the host becomes susceptible (Lambert 1984, Pusey 1989,

and terminal buds are able to produce more shoots from dormant buds on a tree. A tree grows new shoots to replace those lost in a manner similar to a fire (USDA Forest Service 1991). Shoots are often attacked by *Arctia*.

Some trees develop a large root burl (cankers) which allows them to be damaged by fire or disease. Buds used in resprouting are located at the root collar and grow into trees that have lost much of their crown. Other fungi and dieback pathogens cause buds to be damaged. In some species, buds are not damaged by fire. The root collar of madrone trees contains a burl, which contains a large amount of stored energy. Some species allocate more energy to the burl than do other species. Madrone trees have a well-developed root collar and spreading lateral roots (Lambert 1991). These large trees are often attacked by birds (Raphael 1987).

Some individuals of early successional species that establish after a disturbance show decline symptoms before the stand reaches maturity. This phenomenon is known as the "juvenile decline" (Gerrish 1993, Mueller-Landau 1993). A natural part of the species' life cycle. Madrones are more susceptible to decline in an early successional stage. They are adapted to frequent fires (Lambert 1991, USDA Forest Service 1991). Madrones have a growth habit that allows young plants or seedlings, to be



Figure 2. Arthrospores and conidia of *Natrassia mangiferae* taken from a madrone canker. 400x.

competitive in early stages of stand development (Loehle 1987). When these trees approach maturity and senescence, growth slows due to a decreased ratio of photosynthesis to respiration caused by the higher proportion of support tissue relative to leaves. Rapid growth can no longer be used as a defense and the tree is more susceptible to attack by pathogens. As the crown becomes defoliated by pathogens, the amount of photosyn-

thesis relative to respiration decreases even more and opportunistic pathogens, such as *Armillaria*, attack the weakened tree. Water stress on the whole tree increases as the roots become compromised, further weakening the tree. After a certain point, the tree does not have enough carbon in reserve to defend against pathogens and re-grow damaged parts. This process, where the tree has low defenses, is compromised by a short-term stress. The

tree dies due to the activity of secondary organisms in a process called a decline spiral (Manion 1981). Vigorous trees can escape and regrow damaged parts, but trees with low carbon reserves often die.

The objectives of our research were to determine: (1) the role of canker fungi in madrone decline in the Puget Sound area, (2) the fungal composition of cankers and the influence of branch size and distance from the canker margin, and (3) the effects of *Natrassia* and *Fusicoccum* on starch reserves in the root burl.

Methods

Study Sites

The sites chosen for this research were in King County, Washington. All sites were influenced by urbanization and included city parks and other public and private property. The sites ranged from steep, exposed bluffs to flat, protected areas. Soils were generally sandy, but in some areas compacted or waterlogged.

Fungal Composition of Madrone Cankers

Madrone trees growing on four sites in King County, Washington were sampled during the summer of 1997. These sites were Discovery Park, Seward Park, Maury Island, and the University of Washington campus in Seattle. Samples were taken from (1) newly forming cankers without a callus ridge and (2) older cankers with callus and possibly decay on both small (<20 cm) and large diameter stems and branches. Five cankers were sampled from each type. Four wood chips from the phloem, cambium and first few rings of sapwood were removed with a chisel at distances of 0, 0.5, 1, 2, 4, and 8 cm above and below the canker margin. If the branch was dead above the canker, all samples were taken below the canker in living tissue. The wood chips were surface sterilized in a dilute solution of sodium hypochlorite and rinsed in deionized water. They were placed in petri dishes containing 2% malt extract agar and incubated at 25°C for one week. The number of wood chips containing the canker fungi *N. mangiferae* and *F. aesculi* were counted. Other fungi, including yeasts, and bacteria were noted. Isolates of *N. mangiferae* from Seattle were compared to two isolates from the culture collection at the University of Alberta Microfungus Collec-

tion and Herbarium (isolates UAMH 6799 and UAMH 6800). *Natrassia mangiferae* isolates from madrone were also compared with descriptions in the literature (Wilson 1949, Sigler and Carmichael 1976, Sutton and Dyko 1989). Fungi thought to be *F. aesculi* were isolated and compared with descriptions in the literature (English et al. 1974, Pennycook and Samuels 1985, Rayachhetry et al. 1996, Gardner 1997). It was not possible to obtain a culture of this fungus from a culture collection.

Two-way ANOVA was used to compare isolation frequencies of canker fungi in the different canker types on large and small diameter wood. Isolation frequencies of the fungi at different distances from the canker margin were also compared. The Tukey multiple comparison test was used to examine means which were significantly different at the $P=0.05$ level (Neter et al. 1990).

Koch's postulates were tested with a randomly selected isolate of *N. mangiferae* on 24 two-year old madrone seedlings grown in 24 cm diameter pots containing unfertilized vermiculite and peat. They were kept in a glass shelter under outdoor conditions of light and temperature at the University of Washington. A 1 cm cut was made into the sapwood about halfway up the stem of each plant. Four of the plants served as controls, with a sterile toothpick inserted into the wound. The other plants were inoculated with toothpicks colonized by *N. mangiferae*. After each plant was treated, the wound was wrapped in parafilm. The toothpicks and parafilm were removed after 2 wk. Lesions were later sampled and *N. mangiferae* was re-isolated from inoculated plants.

Effects of Disease on Stored Starch

Two hundred fifty-eight madrone trees growing on six sites in King County were surveyed during summer 1997. The sites were three Seattle city parks (Magnolia Bluffs, Seward, and Lincoln), the University of Washington campus, the Crossroads area of Bellevue, and the Northgate area of Seattle. All sites were in urban areas and ranged from lightly used areas with the madrones growing mixed with other tree species and native vegetation in greenbelts to heavily disturbed, where the madrones were part of a landscape that included lawn, ornamental plantings, or concrete. Information was collected on madrone health including amount of cankering and defoliation from branch dieback.

Percent foliage (%FOL) was calculated using the following formula to give an estimate of the tree's percent foliage remaining:

$$\%FOL = [LCR - (LDCR)] / LCR \times 100$$

where LCR = live crown ratio, the ratio of the crown with live foliage to the total crown of the tree, and LDCR = live crown ratio of diseased trees, the base of the crown where the tree was healthy based on diameter (dbh) was also used to estimate the amount of cankering on the main stem. The amount of cankering was determined by classifying the tree into groups based on the number of cankers: group 0, no cankers; group 1, 1-5 cankers; group 2, 6-15 cankers; and group 3, >15 cankers.

A subset of 55 trees from the study was selected for starch analysis in February 1998.

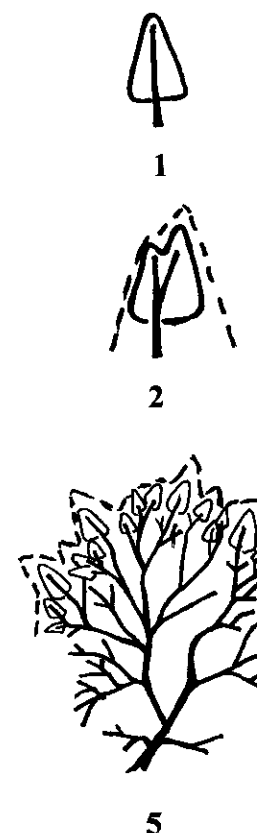


Figure 3. Architecture Based on shoot length. As the shoot length increased, the tree's architecture changed from a simple upright tree (1) to a more complex, bushy structure (2) and finally to a very dense, multi-stemmed structure (5) as the shoot length increased at the top of the tree.

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Starch in Stored Starch

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Percent foliage (%FOL) was calculated from the following formula to get a more realistic measurement of the tree's photosynthetic ability

$$\%FOL = [LCR - (LCR * DEAD)] * 100$$

where LCR = live crown ratio and DEAD = fraction of the crown with branch dieback. On heavily diseased trees, the base of the live crown was estimated to be where live foliage would begin if the tree was healthy based on branching patterns. Diameter (dbh) was also measured. The amount of cankering on the main stem and branches was determined by classifying trees into three groups based on the number of cankers. Group 1 trees contained 0 - 5 stem cankers, group 2, 5 - 15 cankers, and group 3, >15 cankers.

A subset of 55 trees from these sites was used for starch analysis in February 1997. They were

classified using crown architecture as a measurement of physiological age with a seven stage Architecture Based Classification (ABC) (Gatsuk et al. 1980, Mayer 1998) (Figure 3). Stored starch in the lignotuber represented an objective index of tree vigor and a measurement of the resources available after respiration.

In addition to these trees, a second starch sampling was performed on a group of mature madrone trees of similar age and size at the Lincoln Park site in February, 2000. They were separated into groups of healthy ($\geq 50\%$ foliage) and unhealthy ($< 50\%$ foliage) trees.

Samples from each madrone tree were taken with an increment borer at four locations around the root burl. The first 1 cm of sapwood inside the phloem was used for each sample. The samples were placed on dry ice immediately after removal

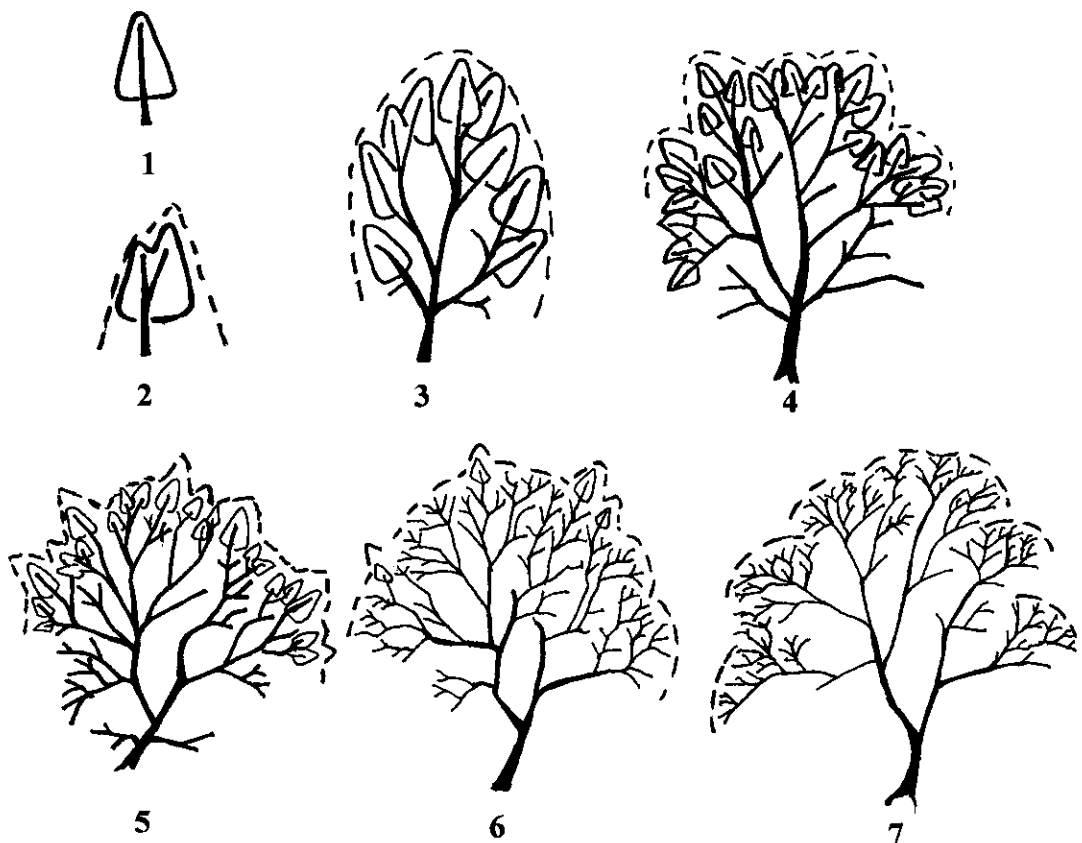


Figure 3. Architecture Based Crown classification (ABC) for Pacific madrone representing physiological age. Stage 1 is the juvenile stage with one central axis. There is increased shoot growth in stages 2 - 5, decreasing as the trees grow in size. As the shoot length decreases, the number of shoots increases. The trees develop a flatter, less dense canopy concentrated at the top of the crown in later stages (6 and 7).

from the tree and then kept at -20°C until analysis. They were dried for 48 hr at 70°C , then ground to a powder for starch analysis. We assumed that starch was stored in the ray cells and inter-ray xylem, and would be present throughout the whole core, since Pacific madrone is a resprouting species (Bell and Ojeda 1999). Thus, the diameter of the tree was irrelevant.

Stored starch was determined using methods modified from Haissig and Dickson (1979) and Kolb and McCormick (1991). Fifty mg of the ground sample was extracted three times with 80% ethanol. The pellet was dried overnight at 70°C . The next day, it was resuspended in acetate buffer at pH 4.5, gelled for 15 min in boiling water, and cooled in an ice bath. Starch was converted to glucose by adding 1 ml of amyloglucosidase (15 units/ml) and incubating for 24 hr at 50°C . After incubation, the samples were removed from the oven and 0.1 ml of sample was added to a 0.4 ml buffer. Five ml of enzyme color reagent (Sigma kit #510) was added to each tube and incubated for 30 min in a 37°C water bath. Absorbance for each sample was read in a Perkin-Elmer 55E spectrophotometer at 450 nm. The amount of glucose in the sample was calculated from a glucose standard regression curve.

The data from the Lincoln Park site were analyzed by t-tests on groups of healthy (percent foliage $\geq 50\%$) and unhealthy (percent foliage $< 50\%$) trees with respect to starch content, dbh, live crown ratio, percent dead branches, and percent foliage. Stored starch was expressed as mg glucose/g sample. The Mann-Whitney U test (Neter et al 1990) was used to determine the differences in the frequency of stem cankers in healthy and unhealthy trees.

Relationships between ABC crown class and other variables and the number of stem cankers and other variables were examined using one-way ANOVA on the data from the other sites. The Tukey multiple comparison test was used to examine means that were significantly different at the $P=0.05$ level (Neter et al. 1990). Data were analyzed using SPSS v. 10.0.

Results

Fungal Composition of Madrone Cankers

Natrassia mangiferae, *Fusicoccum aesculi*, and *Trichoderma* spp. occurred the most frequently

in culture. *Natrassia mangiferae* was found more often than any other species in all cases, and was found in samples from the canker margin 90% of the time. The fungi *Fusicoccum aesculi* and *Trichoderma* spp. were isolated from about 10% of the samples. In some cases, growth of *Trichoderma* suppressed that of *Natrassia*.

Other fungi present in smaller numbers were *Penicillium* spp., *Fusarium* spp., and *Aspergillus* spp., and yeasts. Bacteria were also present.

Microscopic examination showed no morphological difference between isolates of *N. mangiferae* from Pacific madrone cankers in Seattle and isolates from madrone cankers in British Columbia that were in the University of Alberta culture collection. The main identifying feature of *N. mangiferae* is the production of pigmented arthrospores (Figure 2), which distinguishes it from *F. aesculi*. Both of these fungi produce dark mycelia in culture. The isolates from Pacific madrone in Seattle also conform to the descriptions of *N. mangiferae* in the literature (Sutton and Dyko 1989). In the test of Koch's postulates, all the trees inoculated with *N. mangiferae* developed cankers that contained the fungus. These cankers were growing vertically along the stem and were smooth and black, similar to newly developing cankers found on stressed madrone trees in the field.

The number of organisms isolated decreased with distance from the canker margin. In some samples, especially those taken farthest from the canker margin, no fungi were isolated. The number of samples containing *N. mangiferae* declined significantly ($P < 0.001$) with distance from the canker margin. There was a ~50% decrease between 0 and 0.5 cm (Figure 4). The fungus was found in only 21% of the samples at the distance of 8 cm.

Differences between canker types were significant for *N. mangiferae*, with the fungus found most often in actively growing cankers on large diameter wood (Figure 5) ($P = 0.03$). *Natrassia mangiferae* was found least often in cankers on small-diameter branches. There was no significant difference in the amount of *N. mangiferae* found in new and old cankers, and in cankers on stems vs. branches.

Fusicoccum aesculi did not occur in as many samples as *N. mangiferae*, but was found most frequently in cankers on small-diameter branches

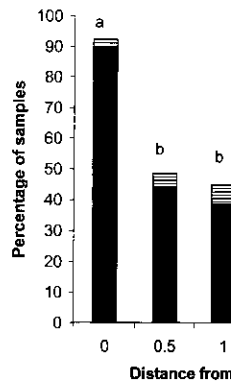


Figure 4. Isolation frequency of *Natrassia mangiferae* and other fungi from madrone cankers at different distances from the canker margin. The height of each bar indicates the percentage of samples containing the fungus. Letters above the bars indicate significant differences between distances.

(Figure 5). It was found most often at a distance of 1 cm from the canker margin for *Natrassia mangiferae*, which was found in 21% of the samples at the canker margin, as compared to 10% on almond trees.

There were no significant differences between the canker types and the amount of *N. mangiferae* found in the canker margin for *Trichoderma* spp. and *Fusicoccum aesculi*. These cankers tended to be older than newer cankers. There were no significant differences between the amount of *N. mangiferae* found in the canker margin and the amount of *Fusicoccum aesculi* in the canker margin.

Effects of Disease on Tree Growth

Large diameter trees showed a significant decrease in age in the crown and a significant increase in diameter trees. The amount of foliage in the crown increased with distance from the main stem (Table 1).

Stored starch was found in 3 through 6 and low

mangiferae was found more species in all cases, and was the canker margin 90% of *Fusicoccum aesculi* and *Trichoderma* spp. isolated from about 10% of samples, growth of *Trichoderma* spp. was also present.

Isolation in smaller numbers were *Trichoderma* spp., and *Aspergillus* spp. were also present.

Isolation showed no morphological differences between isolates of *N. mangiferae* from cankers in Seattle and isolations from cankers in British Columbia. The diversity of Alberta culture collections is a distinguishing feature of *N. mangiferae*. The production of pigmented arthrospores which distinguishes it from other fungi produce dark mycelia. Isolations from Pacific madrone in the field are similar to the descriptions of *N. mangiferae* in the literature (Sutton and Dyko 1974). According to Koch's postulates, all the trees with cankers developed cankers when inoculated with *N. mangiferae*. These cankers were smooth and grew along the stem and were smooth on newly developing cankers on Pacific madrone trees in the field.

The number of organisms isolated decreased with distance from the canker margin. In some cases those taken farthest from the canker margin were isolated. The number of *N. mangiferae* declined with distance from the canker margin (Figure 4). There was a ~50% decrease between the 0 and 1 cm distances (Figure 4). The fungus was found in 50% of the samples at the distance

between canker types were significant differences, with the fungus found in older growing cankers on large diameter trees ($P = 0.03$). *Natrrassia mangiferae* was found least often in cankers on stems and branches. There was no significant difference in the amount of *N. mangiferae* isolated in cankers on stems and branches, and in cankers on

Fusicoccum aesculi did not occur in as many cankers as *N. mangiferae*, but was found most often on small-diameter branches

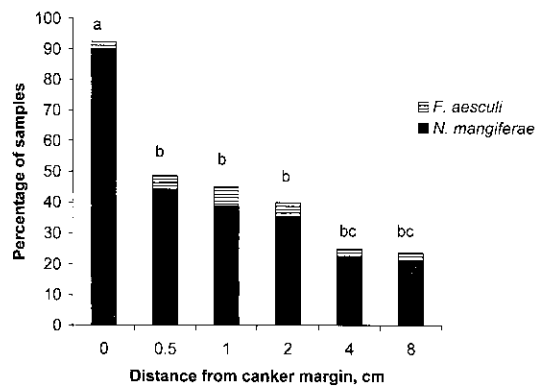


Figure 4. Isolation frequency of the canker fungi *Natrrassia mangiferae* and *Fusicoccum aesculi* from Pacific madrone cankers. Average percentage of isolations out of 20 attempted for distance from the canker margin. The highest proportion of isolations was made up to 1 cm from the canker margin. No organisms were isolated from many of the samples at distances greater than 2 cm from the canker margin. There were significant differences in the amount of *Natrrassia mangiferae* related to distance from the canker margin ($P < 0.01$). Letters above each bar indicate significant differences in the amount of *Natrrassia* isolated. There were no significant differences in the amount of *Fusicoccum* isolated.

(Figure 5). It was found most often at the distance of 1 cm from the margin, as opposed to *N. mangiferae*, which occurred most often at the canker margin (Figure 4). It occurred in the same canker as *Natrrassia* on several samples taken from canker margins, as noted by English et al. (1974) on almond trees.

There were no significant differences among the canker types and distances from the canker margin for *Trichoderma* spp., however, more *Trichoderma* spp. was observed in older cankers. These cankers tended to have less *N. mangiferae* than newer cankers. There was no relationship between the amount of *Trichoderma* spp. and *F. aesculi* in the cankers.

Effects of Disease on Stored Starch

Large diameter trees tended to have less live foliage in the crown and more stem cankers than small diameter trees. The amount of branch dieback in the crown increased with the number of cankers on the main stem (Table 1).

Stored starch was highest in ABC crown classes 3 through 6 and lowest in young trees (crown

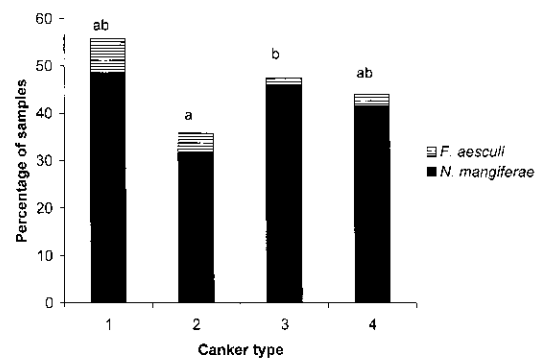


Figure 5. Isolation frequency of the canker fungi *Natrrassia mangiferae* and *Fusicoccum aesculi* from Pacific madrone cankers. Average percentage of isolations out of 20 attempted for each canker type. The canker types sampled were the following: 1 = new cankers on branches < 20 cm diameter, 2 = old callusing cankers on branches, 3 = new cankers on stems and branches > 20 cm diameter, and 4 = old callusing cankers on stems. Five cankers were sampled for each type, with four samples taken from each location. There were significant differences in the amount of *Natrrassia mangiferae* related to each canker type ($P < 0.05$).

classes 1 and 2) and in the oldest trees (crown class 7) (Table 2). Mean diameter of the trees in each group increased and the percentage of foliage decreased, with a large decrease in foliage between crown classes 6 and 7. There was a similar decrease in starch content between these two groups, suggesting that >50% defoliation affects the amount of stored starch in mature trees.

In a selected group of large diameter trees (mean dbh 68 cm) at the Lincoln Park site, healthy trees ($\geq 50\%$ foliage) had fewer stem cankers than unhealthy trees (< 50% foliage) (Figure 6). Healthy mature madrones had more stored starch than

TABLE 1. Number of *Natrrassia* cankers on the main stem and large branches of Pacific madrone relative to mean stem dbh and branch dieback. Standard error in parentheses. Different letters within columns indicate significant differences. ($P < 0.05$).

Number of cankers/tree (number of trees)	dbh(cm)	Branch dieback (%)
0 - 5 (86)	38.2 ^a (2.3)	16.1 ^a (2.5)
5 - 15 (32)	46.3 ^b (2.6)	29.9 ^b (3.4)
>15 (25)	48.9 ^b (1.8)	54.2 ^c (2.7)

TABLE 2. Summary of data for madrones in each ABC (Architecture Based Crown Class). Stored starch is expressed in terms of mg glucose/g sample. Numbers followed by a different letter in each row are significantly different ($P < 0.05$).

ABC	1	2	3	4	5	6	7
Number of trees	2	7	5	8	13	18	7
dbh (cm)	5.0 ^a	5.4 ^a	32.2 ^{ab}	40.4 ^b	39.6 ^b	59.3 ^b	59.0 ^b
% dead	0 ^c	0 ^a	2.0 ^a	2.5 ^a	10.5 ^a	17.1 ^{ab}	51.7 ^b
% foliage	100 ^a	100 ^a	77.7 ^{ab}	73.1 ^{ab}	40.4 ^{bc}	41.9 ^{bc}	12.2 ^c
Starch (mg/g)	49.3 ^{ab}	57.7 ^{ab}	77.9 ^c	65.5 ^{abc}	65.7 ^{abc}	68.4 ^{bc}	46.7 ^a

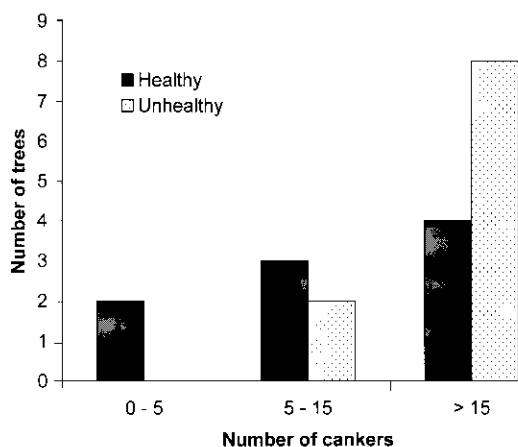


Figure 6. Number of stem cankers on a selected group of large diameter trees (mean dbh 68 cm). Healthy trees (> 50% foliage) had fewer stem cankers than unhealthy trees (< 50% foliage) ($P = 0.08$, Mann-Whitney U test).

TABLE 3. Results of t-tests between healthy ($\geq 50\%$ foliage) and unhealthy ($< 50\%$ foliage) on mature madrone trees at Lincoln Park, Seattle, Washington for dbh, live crown ratio (LCR), % crown dieback, % foliage, and stored starch. Standard error in parentheses.

	Healthy	Unhealthy	P
dbh (cm)	58.3 (22.5)	54.3 (15.7)	0.66
LCR	0.80 (0.04)	0.77 (0.26)	0.92
% crown dieback	27 (6)	78 (4)	<0.01
% foliage	50 (4)	13 (3)	<0.01
Stored starch (mg/g)	2.22 (1.04)	1.18 (0.70)	0.02

unhealthy trees (Table 3). Starch levels were generally lower in this group than those shown in Table 2 because the trees were sampled in February 2000 when the average temperature for November through February was 6.7°C. This was a warmer winter than 1997, when the first group

of starch samples was taken, when the average temperature was 5.3°C (Western Regional Climate Center 2002). The higher temperature in 2000 may have resulted in more growth and less starch storage in Pacific madrones, since they are an evergreen species and can photosynthesize during the winter if conditions are favorable.

Discussion

Nattrassia mangiferae appears to be an introduced fungus and is causing considerable dieback and mortality in Pacific madrone in western Washington. Low disease resistance in madrone, severe weather conditions, and the practice of fire suppression in the 20th century may have made this typically weak pathogen a serious problem on Pacific madrone. Greater weakening of trees through defoliation is caused by the opportunistic pathogen *Fusicoccum aesculi*.

Without fire to destroy inoculum, disease can increase in a stand of Pacific madrones. Above-ground parts are killed by disease rather than fire. Infection by *N. mangiferae* and *F. aesculi* may also reduce fruit and seed production by killing fruit-producing twigs in the upper crown. Declining trees are less able to resprout after the aboveground portion of the tree is killed by disease since the root burl is depleted of starch. It appears that disease is replacing fire as the main disturbance agent responsible for killing aboveground plant parts.

Stored starch decreases with physiological age, reducing reserves to defend against disease and predisposing the trees to decline (Table 2). This trend was also seen in the group of mature madrones sampled at Lincoln Park and is similar to forest declines in which the dominant and codominant trees in a stand have the most severe decline symptoms (Manion, 1981, Gerrish 1993, Mueller-Dombois 1993). This pattern is common in species

that establish after a disturbance. Species that are shade intolerant (Ogden 1993) have a diameter for open-growth that is around 40 cm dbh. Trees with dbh >40 cm dbh is in balance between photosynthesis and demand. A tree must be able to lose some of its foliage to maintain a balance of photosynthetic to respiratory demand for a tree to begin to decline (Ogden et al. 1993). Larger, older trees are unable to defend themselves against disease or growth or defensive costs that they must require carbon.

Defoliation as a result of disease and other fungi can add to the decline of a mature madrone tree. *Nattrassia mangiferae* girdling the stem creates a wound that leads to the death of the tree. Under these conditions, the pathogen *Fusicoccum aesculi* attacks the twigs and branches. A dieback is created when the tree is unable to maintain its canopy to branch dieback. In mature trees drop significantly. Trees with less than 50% foliage (Table 3) and 3). Trees with >50% foliage have difficulty escaping the disease because of insufficient carbohydrates available for defense because their energy reserves are lessened. Changes in crown structure of defoliated madrones make them more susceptible to other pathogens (Garraway et al. 1991). *Armillaria* would create a wound that further reducing tree defense. *Armillaria* age by canker fungi. *Armillaria* metabolism in declining trees. This study was conducted to confirm the

Literature Cited

- Adams, A. B., F. J. Harvey, W. J. G. Adams, and J. A. Adams. 1977. Habitat physical structure and the decline of *Armillaria menziesii* in a Seattle forest. *Forest Pathology* 77. In A. B. Adams and J. A. Adams (eds.), *The Decline of the Pacific Northwest Forest* (Pursh): Current Theories and Research. Proceedings of the April 1977 Symposium, the Center for Urban Horticulture, University of Washington, Seattle. Research papers. Save the Redwoods Society, Urban Horticulture, Environment and Research, Seattle.

red starch is expressed in terms of significantly different ($P < 0.05$).

	6	7
	18	7
	59.3 ^b	59.0 ^b
	17.1 ^{ab}	51.7 ^b
	41.9 ^{bc}	12.2 ^c
	68.4 ^{bc}	46.7 ^a

s taken, when the average (Western Regional Climate warmer temperature in 2000 may growth and less starch storage since they are an evergreen photosynthesize during the winter favorable.

appears to be an introduced considerable dieback and madrone in western Washington resistance in madrone, seasons, and the practice of fire century may have made pathogen a serious problem Greater weakening of trees caused by the opportunistic *Fusicoccum aesculi*.

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that establish after a disturbance such as fire and are shade intolerant (Ogden et al. 1993). The critical diameter for open-grown or codominant madrones seems to be around 40 cm. A madrone tree that is >40 cm dbh is in balance between carbon production and demand. A stress that causes the tree to lose some of its foliage will decrease the ratio of photosynthetic to respiring area and cause the tree to begin to decline (Gerrish 1993, Ogden et al. 1993). Larger, older trees lack the resources to defend themselves against canker fungi by growth or defensive chemicals, both of which require carbon.

Defoliation as a result of the presence of canker fungi can add to the effects of age on a mature madrone tree. *Natrrassia mangiferae* cankers girdling the stem create water stress in the crown. Under these conditions, the opportunistic pathogen *Fusicoccum aesculi* becomes active and kills twigs and branches. A decline spiral (Manion 1981) is created when the trees have lost >50% of their canopy to branch dieback. Stored starch levels in mature trees drop significantly when compared to trees with less than 50% defoliation (Tables 2 and 3). Trees with >50% defoliation may have difficulty escaping the decline spiral without sufficient carbohydrates available for growth and defense because their photosynthetic ability is lessened. Changes in carbohydrate chemistry in defoliated madrones may also reduce their resistance to other pathogens, such as *Armillaria* (Garraway et al. 1991). Increased infection by *Armillaria* would create more water stress, further reducing tree defenses and increasing damage by canker fungi. A study of carbohydrate metabolism in declining madrones needs to be conducted to confirm this.

Literature Cited

- Adams, A. B., F. J. Harvey, W. T. Crooks, and P. Wilson. 1999. Habitat physical structure and status of *Arbutus menziesii* in a Seattle urban environment. Pages 61-77 In A. B. Adams and C. W. Hamilton, (editors), *The Decline of the Pacific Madrone (Arbutus menziesii Pursh): Current Theory and Research Directions*. Proceedings of the April 28, 1995 Symposium held at the Center for Urban Horticulture and subsequent research papers. Save Magnolia's Madrones, Center for Urban Horticulture. Ecosystems Database Development and Research, Seattle, Washington.

Small-diameter branches in the upper crown of madrone trees exhibit symptoms of dieback before large-diameter branches and the main stem. During drought periods small-diameter branches are the first to undergo water stress, causing *Fusicoccum aesculi* to become aggressive (Houston 1984, Boyer 1995). Additional water stress due to *N. mangiferae* cankers lower on the tree can also decrease the water supply to the upper branches. *Fusicoccum* was not isolated as often as *Natrrassia* from cankers in our study. *Fusicoccum* may cause more damage to madrones in California than in western Washington and British Columbia because of the drier climate there. In California, *Fusicoccum* produces wedge-shaped cankers on madrone (McDonald and Tappeiner 1991), but we did not observe these. However, all the declining trees had *Fusicoccum* dieback in the crown. We tended not to sample these branches and concentrated our sampling on typical *Natrrassia* cankers that occur more commonly on large diameter material. *Fusicoccum* could be more important than our sampling indicated. Although *Fusicoccum* may cause more damage on madrones in California, in western Washington and British Columbia *Natrrassia* is the primary canker pathogen on larger diameter material. *Trichoderma* is not known to be pathogenic and may be competing successfully for space and nutrients with *Natrrassia* in older cankers.

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- Adams, D., and K. Kosta. 1993. Madrone canker in California. Tree Notes No. 16. California Department of Forestry and Fire Protection, Sacramento, California.
- Bell, T. L., and F. Ojeda. 1999. Underground starch storage in *Erica* species of the Cape Floristic Region—differences between seeders and resprouters. *New Phytologist* 144:143-152.
- Boyer, J. S. 1995. Biochemical and biophysical aspects of water deficits and the predisposition to disease. *Annual Review of Phytopathology* 33:251-74.
- Bressette, D. K. 1995. Determining causes of decline of Pacific madrone in urban landscapes of the Pacific Northwest.

- M.S. Thesis, University of Washington, Seattle, Washington.
- Calavan, E. C., and J. M. Wallace. 1954. *Hendersonula toruloidea* Nattrass on citrus in California. *Phytopathology* 44:635-639.
- Davison, A. D. 1972. Factors affecting development of madrone canker. *Plant Disease Reporter* 56:50-52.
- English, H., J. R. Davis, and J. E. DeVay. 1974. Relationship of *Botryosphaeria dothidea* and *Hendersonula toruloidea* to a canker disease of almond. *Phytopathology* 65:114-122.
- Gardner, D. E. 1997. *Botryosphaeria mamane* sp. nov. associated with witches'-brooms on the endemic forest tree *Sophora chrysophylla* in Hawaii. *Mycologia* 89:298-303.
- Garraway, M. O., A. Huttermann, and P.M. Wargo. 1991. Ontogeny and physiology. Pages 21-47 *In* G. G. Shaw, III and G. A. Kile (editors), *Armillaria Root Disease*. USDA Forest Service Agriculture Handbook No. 691. Washington, D. C.
- Gatsuk, L. E., Smirnova, O. V., Vorontzova, L. B., and L. A. Zhukova. 1980. Age states of plants of various growth forms: a review. *Journal of Ecology* 68:675-696.
- Gerrish, G. 1993. Using a life history-carbon balance model for forest decline research. Pages 243-250 *In* R. F. Huettl and D. Mueller-Dombois (editors), *Forest Decline in the Atlantic and Pacific Region*. Springer-Verlag, Berlin.
- Haissig, B. E., and D. E. Dickson. 1979. Starch measurement in plant materials using enzymatic hydrolysis. *Physiologia Plantarum* 47:151-157.
- Houston, D. R. 1984. Stress related to diseases. *Arboricultural Journal* 8:137-149.
- Hunt, R. S., B. Callan, and A. Funk. 1992. *Common Pests of Arbutus*. Canadian Forestry Service, FPL 63, Victoria, British Columbia.
- Jayasingh, C. K., and W. P. K. Silva. 1994. Foot canker and sudden wilt of *Hevea brasiliensis* associated with *Natrassia mangiferae*. *Plant Pathology* 43:938-940.
- Kolb, T. E., and L. H. McCormick. 1991. Relationship between total nonstructural carbohydrate concentration and root diameter in sugar maple. *Forest Science* 37:343-346.
- Loehle, C. 1987. Tree life history strategies: the role of defenses. *Canadian Journal of Forest Research* 18:209-222.
- Manion, P. D. 1981. *Trec Disease Concepts*. Prentice Hall, Englewood Cliffs, New Jersey.
- Mayer, S. 1998. Architecture Based Classification (ABC): Evaluation of an architectural method designed to assess vigor of Pacific madrone. Unpublished Report to Save Magnolia's Madrones. On file at 4751 West Ruffner St. Seattle, Washington 98199.
- McDonald, P. M., and J. C. Tappeiner, II. 1991. *Arbutus menziesii* Pursh Pacific Madrone. USDA Forest Service Agriculture Handbook #654, USDA Forest Service, Washington, D. C. Available online at http://www.na.fs.fed.us/spfo/pubs/silvics_manual/table_of_contents.htm.
- Msikita, W., J. S. Yaniniek, M. Ahounou, H. Baimy, and R. Fagbemissi. 1997. First report of *Natrassia mangiferae* root and stem rot of cassava in West Africa. *Plant Disease* 81:1332.
- Mueller-Dombois, D. 1993. A natural dieback theory, cohort senescence as an alternative to the decline disease theory. Pages 26-37 *In* P. D. Manion and D. Lachance (editors), *Forest Decline Concepts*. APS Press, St. Paul, Minnesota.
- Nattrass, R. M. 1933. A new species of *Hendersonula* (*H. toruloidea*) on deciduous trees in Egypt. *Transactions of the British Mycological Society* 18:189-198.
- Neter, J., W. Wassermann, and M. Kutner. 1990. *Applied Linear Statistical Models: Regression, Analysis of Variance, and Experimental Design*. Richard D. Irwin, Inc. Burr Ridge, Illinois.
- Ogden, J., C. H. Lusk, and M. G. Steel. 1993. Episodic mortality, forest decline, and diversity in a dynamic landscape: Tongariro National Park, New Zealand. Pages 261-274 *In* R. F. Huettl and D. Mueller-Dombois (editors), *Forest Decline in the Atlantic and Pacific Region*. Springer-Verlag, Berlin.
- Pandey, R. S., S. N. Bhargava, D. N. Shukla, and D. V. S. Khati. 1981. A new leaf spot disease of mango. *Plant Disease* 65:441-442.
- Paxton, J. D., and E. E. Wilson. 1965. Anatomical and physiological aspects of branch wilt disease in Persian walnut. *Phytopathology* 55: 21-26.
- Pennycook, S. R., and G. J. Samuels. 1985. *Botryosphaeria* and *Fusicoccum* species associated with ripe fruit rot of *Actinidia deliciosa* (kiwifruit) in New Zealand. *Mycotaxon* 24:445-458.
- Pusey, P. L. 1989. Influence of water stress on susceptibility of nonwounded peach bark to *Botryosphaeria dothidea*. *Plant Disease* 73:1000-1003.
- Raphael, M. G. 1987. Use of Pacific madrone by cavity-nesting birds. Pages 198-202 *In* T. R. Plumb and N. H. Pillsbury (technical coordinators), *Proceedings. Symposium on Multiple Use Management of California's Hardwood Resources*, November 12-14, 1986, San Luis Obispo, California. USDA Forest Service General Technical Report PSW-100. Pacific Southwest Forest and Range Experiment Station, Berkeley, California.
- Rayachhetry, M. B., G. M. Blakeslee, R. S. Webb, and J. W. Kimbrough. 1996. Characteristics of the *Fusicoccum* anamorph of *Botryosphaeria ribis*, a potential biological control agent for *Melaleuca quinquenervia* in South Florida. *Mycologia* 88:239-248.
- Sigler, L., and J. W. Carmichael. 1976. Taxonomy of *Malbranchea* and some other hyphomycetes with arthroconidia. *Mycotaxon* 4:349-488.
- Sigler, L., R. C. Summerbell, L. Poole, M. Wieden, D. Sutton, M. G. Rinaldi, M. Aguirre, G. W. Estes, and J. N. Galgiani. 1997. Invasive *Natrassia mangiferae* infections: case report, literature review, and taxonomic appraisal. *Journal of Clinical Microbiology* 35:433-440.
- Sutton, B. C., and B. J. Dyko. 1989. Revision of *Hendersonula*. *Mycological Research* 93:466-488.
- USDA Forest Service. 2002. Fire effects information system. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory, Missoula, Montana. Available online at <http://www.fs.fed.us/database/feis/>.
- Wilson, E. E. 1947. The branch wilt of walnut and its cause. *Hilgardia* 1:1-12.
- Wilson, E. E. 1949. The pycnidial wilt fungus, *Exosporium* sp. nov. *Phytopathology* 39:705-712.

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of cassava in West Africa. *Plant Dis-*

1993. A natural dieback theory, cohort
an alternative to the decline disease
37 In P. D. Manion and D. Lachance
Decline Concepts. APS Press, St. Paul,

A new species of *Hendersonula* (*H.*
aciduous trees in Egypt. *Transactions*
Ecological Society 18:189-198.

and M. Kutner. 1990. *Applied Linear*
s: Regression, Analysis of Variance,
I Design. Richard D. Irwin, Inc. Burr

and M. G. Steel. 1993. Episodic mor-
tality, and diversity in a dynamic land-
National Park, New Zealand. Pages
Huettl and D. Mueller-Dombois (edi-
line in the Atlantic and Pacific Re-
verlag, Berlin.

argava, D. N. Shukla, and D. V. S.
ew leaf spot disease of mango. *Plant*
42.

Wilson. 1965. Anatomical and physi-
of branch wilt disease in Persian wal-
ogy 55: 21-26.

J. Samuels. 1985. *Botryosphaeria*
species associated with ripe fruit rot
cinosa (kiwifruit) in New Zealand.
45-458.

ence of water stress on susceptibility
peach bark to *Botryosphaeria*
disease 73:1000-1003.

of Pacific madrone by cavity-nesting
02 in T. R. Plumb and N. H. Pillsbury
ators), Proceedings, Symposium on
agement of California's Hardwood
mber 12-14, 1986, San Luis Obispo,
A Forest Service General Technical
Pacific Southwest Forest and Range
on, Berkeley, California.

A. Blakeslee, R. S. Webb, and J. W.
. Characteristics of the *Fusicoccum*
osphaeria ribis, a potential biologi-
or *Melaleuca quinquenervia* in South
ia 88:239-248

Carmichael. 1976. Taxonomy of
d some other hyphomycetes with
cotaxon 4:349-488.

ell, L. Poole, M. Wieden, D. Sutton,
I. Aguirre, G. W. Estes, and J. N.
vasive *Natrassia mangiferae* infec-
literature review, and taxonomic ap-
Clinical Microbiology 35:433-440.

ko. 1989. Revision of *Hendersonula*.
arch 93:466-488.

02. Fire effects information system.
f Agriculture. Forest Service, Rocky
h Station, Fire Sciences Laboratory,
ana. Available online at [http://
abase/feis/](http://database/feis/).

Wilson, E. E. 1947. The branch wilt of Persian walnut trees
and its cause. *Hilgardia* 17:413-430.

Wilson, E. E. 1949. The pycnidial stage of the walnut branch
wilt fungus, *Exosporina fawcettii*. *Phytopathology*
53:705-712.

Received 11 February 2002

Accepted for publication 5 July 2002

Western Regional Climate Center. 2002. Desert Research In-
stitute, Reno, Nevada. Available online at [http://
www.wrcc.sage.dri.edu/](http://www.wrcc.sage.dri.edu/).