DISEASES THREATEN THE SURVIVAL OF IONE MANZANITA (*ARCTOSTAPHYLOS MYRTIFOLIA*)

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In fulfillment of subcontract for:
Conservation and Recovery of Ione Endemic Plants

Supported by a prime contract awarded to San Francisco State University by the **California Department of Fish and Game**

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March 10, 2003
SFSU Agreement No. C7-94070
Phytosphere Research No. 2001-1202

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Cover photos: Top, *Fusicoccum* canker in *A. myrtifolia*, December 2001. Bottom, mortality center caused by *P. cinnamomi* at Apricum Hill Preserve, October 2002.

ACKNOWLEDGEMENTS

We thank Tiffany Meyer for inviting us to participate in this study. Tiffany provided assistance with fieldwork and acquired the *A. visicida* plants and rooted the *A. myrtifolia* cuttings used for pathogenicity tests. Burt Johnson and Steve Edwards at Tilden Park provided space and assistance to Tiffany in propagating the cuttings of *A. myrtifolia*.

George Hartwell alerted us to the scale of the die-off problem in *A myrtifolia* by pointing out numerous areas where large patches of plants were killed.

Greg Browne, Tom Gordon, and Dave Rizzo, Plant Pathology Dept., UC Davis, provided support and cooperation by allowing us to use lab facilities and providing specialized culture media.

Matteo Garbelotto's lab at the Department of Environmental Science, Policy, and Management, UC Berkeley, performed the gene sequencing to identify our *Phytophthora* isolate as *P. cinnamomi*.

Tim Tidwell, California Department of Food and Agriculture, provided a culture of *Fusicoccum aesculi* isolated from *Sequoia sempervirens* in Elk Grove for comparison purposes.

Funding for this project was provided by Phytosphere Research and SFSU Agreement No. C7-94070, a subcontract of interagency agreement P0020015 awarded to San Francisco State University by the California Department of Fish and Game.

EXECUTIVE SUMMARY

We have determined that at least two different diseases are affecting the health of *A. myrtifolia* and *A. viscida* in the Ione area. The first is a branch canker disease caused by species of *Fusicoccum,* which has previously been identified on *A. myrtifolia*. We isolated two species of *Fusicoccum* from stem cankers and completed proof of pathogenicity on both *A. myrtifolia* and *A. viscida*. Most of the *Fusicoccum* cankers that we monitored expanded very little between March and October. Progressive dieback associated with *Fusicoccum* cankers may sometimes cause the death of *A. myrtifolia* plants. Mortality associated with *Fusicoccum* canker tends to be scattered within affected stands.

The second disease, which is newly identified and documented in this report, is a root and crown rot caused by *Phytophthora cinnamomi*. *P. cinnamomi* root and crown rot causes large contiguous patches of mortality in stands of *A. myrtifolia* and *A. viscida*. Infected plants desiccate rapidly at the onset of hot weather. We isolated *P. cinnamomi* from symptomatic plants at two disjunct mortality centers and also recovered the pathogen from soil collected from one mortality center. We completed greenhouse tests to demonstrate pathogenicity of the *P. cinnamomi* isolates to both *A. myrtifolia* and *A. viscida*.

Although the two diseases have not been previously distinguished, they differ in symptomatology, etiology, and their potential for impact on *A. myrtifolia* and other species. *P. cinnamomi* root and crown rot is by far the more serious disease and has the potential to both eliminate entire *A. myrtifolia* populations and prevent recolonization of infested sites by *A. myrtifolia*. Successful conservation of this species will not be possible unless spread of this disease into noninfested stands can be stopped. Hence, a high priority should be placed on delineating the extent of this pathogen within the range of *A. myrtifolia* and implementing measures to restrict the movement of infested soil and plant materials from areas affected by *P. cinnamomi*.

INTRODUCTION

Significant disease problems have been noted in natural stands of Ione manzanita (*Arctostaphylos myrtifolia*) since at least 1988 (Wood and Parker 1989), but little information has been available about the nature of the disease or diseases responsible for dieback and mortality of this species. Adams (1934) described perennial branch cankers occurring on stems of *A. myrtifolia* and *A. viscida* in the Ione area and thought that the condition was most likely caused by a pathogen, but did not undertake pathology studies to identify possible causal agents. Although Gankin and Major (1964) did not mention disease as a significant factor in their study, Wood and Parker (1989) reported the "sudden and virtually complete die-off of entire stands [of *A. myrtifolia*], observed in the past at several locations in the Ione region". They did not present data on the extent or nature of the problem, but suggested that the sudden death of plants "may be caused at least secondarily by a fungal pathogen" and stated that the concentric pattern of disease development "suggests the mycelial growth of a soil fungal pathogen".

Some years after Wood and Parker's (1989) report, plant pathologists at California Department of Food and Agriculture (CDFA) identified *Fusicoccum aesculi* on branch cankers of *A. myrtifolia*. *F. aesculi* and its usually presumed sexual stage (teleomorph) *Botryosphaeria dothidea* have been associated with cankers of numerous woody trees and shrubs. Brooks and Ferrin (1994) reported on branch dieback of various chaparral species, including *Arctostaphylos glauca,* in southern California that was caused by *F. aesculi,* which they referred to as *B. dothidea*.

The purposes of this study were to (1) examine disease symptoms and disease progression in *A. myrtifolia*, (2) identify pathogens associated with disease, and (3) look for associations between site factors and disease development as part of the diagnostic process. Our initial focus was on branch cankers because *F. aesculi* had already been identified as a pathogen of *A. myrtifolia*. Because cankers caused by this species have been associated with water stress and other types of plant stress (Brooks and Ferrin 1994), we established field plots at the CDFG Apricum Hill Preserve to investigate the relationship between branch canker symptom progression, seasonal water stress, and plant growth.

In the course of our field work at the Apricum Hill Preserve, we discovered a large patch of dead *A. myrtifolia* and *A. viscida* plants that had not been previously reported and which was clearly not present at the time of the Wood and Parker (1989) surveys. The pattern of disease was consistent with that caused by a soil-borne plant pathogen rather than the airborne fungus *F. aesculi*. Upon finding this mortality center, we initiated a second series of studies aimed at identifying the cause of this likely root disease. These investigations led to our identification of *Phytophthora cinnamomi* as the cause of the large patches of *A. myrtifolia* mortality first noted by Wood and Parker (1989).

This report presents the results of our investigation into these two diseases affecting *A. myrtifolia* in the vicinity of Ione, Amador County, California. Because the endangered *A. myrtifolia* is difficult to propagate, many of our studies were carried out using the common sympatric species *A. viscida*. Our field observations indicated that *A. myrtifolia* and *A. viscida* are similarly affected by both branch cankers and *P. cinnamomi* root and crown rot.

METHODS

PLANTS USED FOR GREENHOUSE TESTS

For all pathogenicity tests on *A. viscida*, we used rooted cuttings that were one year old when we obtained them in January 2002. These plants were obtained from Cornflower Farms, Elk Grove, CA by Tiffany Meyer. The rooted cuttings were initially growing in 312 ml bottomless pots (5 x 5 x 12.5 cm). We repotted them into 473 ml plastic drinking cups with drain holes in July 2002.

A. myrtifolia cuttings were collected from the Ione area in August 2001 by Tiffany Meyer and rooted in a sand/perlite mixture in an unheated greenhouse at Tilden Park in Berkeley. More than a year elapsed before root development was sufficient for transplanting. Successfully rooted cuttings were transplanted individually into 250 ml plastic cups with drain holes on 3 October 2002 and transferred to a greenhouse at Vacaville, CA. Except for plants transplanted directly into field soil (described below) these rooted cuttings were transplanted into Park's Seeds Grow Mix (Geo. W. Park Seed Co., Inc., Greenwood, SC) with additional perlite added.

STEM CANKERS

Isolations from field-collected material

Our investigations on stem cankers in *A. myrtifolia and A viscida* began in December 2001. With the assistance of Tiffany Meyer, we collected samples from plants with stem dieback and/or canker symptoms located near Lambert Road north of Ione and in the CDFG Apricum Hill preserve. We examined leaf and stem lesions for possible sporulation with light microscopy. To isolate potential plant pathogens, symptomatic leaves and stems were disinfested by soaking in dilute bleach (0.5% NaOCl) for 10 to 30 seconds. Tissue pieces were then excised from the margins of affected areas and placed on sterile culture media. The media used included PARP (Corn meal agar Difco Laboratories, MI, amended with 10 μg/ml pimaricin, ampicillin, rifampicin, and PCNB) (Erwin and Ribeiro 1996); PARP plus 50 μg/ml hymexazol which is selective for *Phytophthora* and inhibitory to most *Pythium* species; malt extract agar (MEA, Difco Laboratories), which is favorable for *Nattrassia;* and acidified potato dextrose agar (aPDA, Difco Laboratories) which is favorable for *Fusicoccum* as well as many other fungi.

Pathogenicity testing of fungi isolated from stem cankers

All pathogenicity tests reported herein were performed in 2002. Fungi isolated from stem cankers were initially screened for pathogenicity on *A. viscida* in February. A culture of *Fusicoccum aesculi* isolated from *Sequoia sempervirens* in Elk Grove, CA (obtained from T. Tidwell, CDFA) was also included in the initial inoculations. Isolates that were pathogenic to *A. viscida* were tested again in October on both *A. viscida* and *A. myrtifolia*.

A. viscida plants used for pathogenicity tests were about 20 to 25 cm tall at the time of inoculation. We selected branched plants that had at least two shoots and performed one inoculation on each shoot (two inoculations per isolate or control). We cut off a fully-expanded leaf near the tip of each shoot, leaving a wound on the stem about 1.5 mm wide by 2.5 mm long. A 6 mm diameter plug cut from an actively growing culture (6 days old on aPDA) was placed with the aerial mycelium side against the cut. The stem and agar plug were wrapped with Parafilm® to retard drying.

Most *A. myrtifolia* plants used for inoculations were branched and less than 10 cm tall. Inoculation methods were the same as used for *A. viscida*, except that the scar made by leaf

excision was much smaller, about 0.5 mm in diameter (Figure 1). For both *A. myrtifolia* and *A. viscida*, control plants were inoculated with a plug of sterile agar. We used three *A. myrtifolia* plants for each isolate tested and another three plants were used for controls.

Figure 1. Inoculation of rooted *A. myrtifolia* cuttings with agar plugs. Inoculation points were wrapped with Parafilm to retard drying of the agar plug.

Plants were maintained indoors at room temperature (20 to 24 C) for the first 24 to 48 h after inoculation and were then transferred to an unheated greenhouse. Greenhouse temperatures fluctuated diurnally, and ranged between 4 to 7 C and 38 C in both February and October. Plants were watered sparingly after inoculation to allow mild water stress to develop in the February inoculation. In the October inoculation, plants were watered regularly.

In February inoculations, agar plugs and Parafilm were removed from all inoculations after 2 weeks. In the October inoculations, agar plugs and Parafilm were removed after 5 days. Plants were examined periodically after inoculation for symptom development over the next 20 days. As plants died or when the experiment was terminated, stem pieces with symptomatic lesions were placed in 0.5% NaOCl for 20 seconds, drained, and then slices were cut through the margins of affected tissue with a sterile scalpel and placed on acid PDA.

Field plots for monitoring stem canker progress and water stress

Field studies were conducted in 2002. We established two field plots at the Apricum Hill Preserve in late February and early March. The upper plot is located on a moderately steep east-facing slope just below a hilltop at an average elevation of 140 m. The lower plot is located on slightly sloping terrain on a low, flat mound at an average elevation of 122 m and has a southerly aspect. The plots are 12 m by 12 m and are situated in nearly pure stands of *A. myrtifolia* (Figure 2).

Figure 2. Upper (top) and lower (bottom) plots at Apricum Hill Preserve. Flags mark locations of tagged plants. Tape measure in bottom photo marks a transect used for point cover assessments.

We made field observations in February, March, May, June, and October. On 23 February and 13 October, we assessed cover in each plot using the point intercept method along six parallel transects spaced 2 m apart. Cover assessments were made at points spaced 0.5 m apart along each transect. At each point we scored the plant species present and whether the plant was live or dead. For points falling on *A. myrtifolia* and *A. viscida* plants, we also noted whether the point intercepted healthy stems, recently-killed stems (with bright brown leaves), older killed stems (with dark brown, gray, or blackish foliage), or dead defoliated stems.

The plots were subdivided along a 2 m by 2 m grid (36 points total) and the nearest live *A. myrtifolia* to each grid point was tagged and numbered. For all tagged plants, we measured maximum shoot height. We also estimated the amount of total dieback and dieback by age category (recent, older, and defoliated as noted above) for each plant using a 0 to 6 scale (Table 1).

In addition, 10 of the tagged plants in each plot were selected at random and photographed in March, May, and October. An additional 10 tagged plants were selected at random and used for water potential determinations, growth measurements, and canker progression measurements.

Table 1. The 0-6 arcsine-transformed percentage scale used for assessing dieback and defoliation of individual plants.

Rating	Percent range
0	Symptom not seen
1	<2.5%
2	2.5% to <20%
3	20% to < 50%
4	50% to < 80%
5	80% to < 97.5%
6	97.5% to 100%

Midday stem water potentials (SWP) were measured 23 February, 11 May, and 13 October, following methods outlined by Shackel (2002). For each plant evaluated, we selected two shoot tips near the central portion of the plant which arose as directly as possible from a main stem. Each shoot tip was sealed in a clear plastic bag and overbagged with a larger opaque reflective plastic bag. These bags prevent transpiration and excessive heating of leaves. Bags were left in place for at least 1 hour to allow leaf water potential to equilibrate to that of the subtending stem. At the time of the reading, the outer opaque bag was removed and the shoot tip was excised and placed into the pressure chamber while still enclosed in the inner plastic bag. One or two SWP readings were made per plant between about 1300 and 1530 PDT. SWP measurements were made with a pump-up pressure chamber (PMS Instrument Co., Corvallis OR) fitted with a 10.2 cm diameter 40 bar (0.4 MPa) gauge with 1% accuracy full scale.

On the same plants used for SWP measurements, we selected up to two branches with dieback and/or stem cankers and measured the length of necrotic tissue. The selected branches were marked with colored beads strung on UV-resistant plastic cable ties. Canker length measurements were made on 3 March, 11 May, and 13 October.

To track growth of individual shoots we marked additional branches on these plants as described above. Shoot lengths were measured from the shoot tip to a marked branch junction on 2 March and 13 October. In selecting shoot tips for SWP measurements, we avoided branches that had shoots marked for either canker or growth measurements.

We used JMP statistical software (SAS Inc., Cary NC) for data analysis. Unless otherwise indicated, effects or differences are referred to as significant if $p \leq 0.05$. We used linear regression and analysis of variance models to test for associations between continuous outcome and continuous or categorical predictor variables. For continuous variables and 0-6 ratings, we also used analysis of variance (F-tests) or t-tests to test for mean differences between groups or dates. Differences in binary count data from transects were tested using logistic regression.

ROOT DISEASE CENTERS

Monitoring of the mortality center at Apricum Hill

We first observed the root disease mortality center at the Apricum Hill Preserve on 3 March. At that time, we mapped the outline of the affected area using a Garmin GPS76 GPS receiver attached to a boom-mounted external antenna.

We also established three transects in portions of the affected area that included both dead and live plants to observe possible disease spread. Two transects were 20 m long and the third was 16.5 m long. One end of each transect was marked with a small numbered galvanized steel plate (about 7 cm square) on the soil surface held in place by a large nail. We also recorded the azimuth of the transect and the GPS coordinates of the transect origin. Point intercept cover and disease symptom ratings were made at 0.5 m intervals along the transects in the same manner used in the field plots described above. These transects were resurveyed 11 May and 13 October.

Isolations from field-collected material

We collected a few samples from diseased plants when the disease center was initially observed in March. We collected additional samples from recently-killed and dying *A. myrtifolia* and *A. viscida* plants on 3 June. Samples on this later date were collected at both the Apricum Hill Preserve and a separate older mortality center located along SR88 about 1.3 km north-northwest of the Apricum Hill site. Tissue pieces from diseased samples were placed into petri plates containing aPDA, PARP, or PARP with hymexazol.

Soil baiting for Phytophthora

On 11 May, we collected two soil samples from the Apricum Hill mortality center. Samples were collected from the upper 8 to 10 cm of the soil profile near declining or dead *A. viscida* and *A. myrtifolia*. An additional sample was collected near healthy *A. myrtifolia* from a nonsymptomatic area within 50 m from the edge of the mortality center.

We used green pears as baits to assay for the presence of *Phytophthora* spp. in the soil samples. In the first round of baiting, all operations were conducted indoors at temperatures of about 24 to 27 C. On 12 May, two subsamples (200-250 ml) from each soil sample were placed in 473 ml plastic cups and adjusted to approximately field capacity with charcoal-filtered tap water. After 24 h, we placed a green Bartlett pear on the soil in each cup and added additional water to bring the water level to about 2.5 cm above the soil surface. Pears were removed from the water after 48 h, rinsed, and placed on dry paper towels in a plastic box. After 2 days, tissue pieces from symptomatic areas of the pear baits were placed into petri plates containing MEA.

Starting on 22 May, we conducted a second round of baiting with the following modifications. Green D'Anjou pears were used instead of Bartlett pears. One set of subsamples was adjusted to field capacity 48 hr before flooding. The other set of subsamples was flooded without the initial wetting cycle, which is intended to favor sporangium formation. Pear baits were placed on aluminum wire mesh window screen to minimize the amount of contact between baits and floating organic debris. The pre-wetted soil subsamples were held at 13 to 18 C for two days before soil was flooded and pears were added. Pears were then left in contact with the flooded soil for 2 days at 13 to 18 C and 2 additional days at 14 to 21 C. For the directly flooded subsamples, pears were placed in contact with the flooded soil for 4 days at 13 to 18 C. After removal from the soil, both sets of pears were incubated at 24 to 27 C. Tissue pieces from symptomatic areas of the pear baits were placed into petri plates containing PARP plus hymexazol media.

Soil samples used in the pear baiting experiments were allowed to dry. Soil samples were then rewetted to saturation for pH and electrical conductivity determinations.

Transplanting *A. viscida* and *A. myrtifolia* into field soil

On 12 May we transplanted three *A. viscida* plants into each of three large pots (3.8 L) that were then filled with one of the three soil samples collected on 11 May. The ratio of field soil to root ball soil of the *A. viscida* transplants was approximately 1:1. Pots were kept well watered and

incubated in the greenhouse. After 6 weeks, we subjected the plants to a 13 h flooding treatment. The experiment was terminated after 7.5 weeks on 2 July. Soil was washed from the root systems and plants were examined and photographed. Tissue pieces from symptomatic roots and lower stems were placed into petri plates containing PARP and PARP plus hymexazol.

On 3 October, we transplanted bare-rooted cuttings of *A. myrtifolia* individually into 250 ml plastic drinking cups (with drain holes) containing field soil. We used two of the soil samples collected 11 May (one each from the mortality center and the healthy area) and another sample from the Apricum Hill mortality center that was collected near the base of a recently-killed *A. viscida* on 3 June. Four cups were used for each soil sample. Plants were held in the greenhouse and watered to keep the soil relatively moist. After 11 days, the cups were nested in larger cups that lacked drain holes and the soil was flooded for 12 h. Observations continued for 4 weeks after transplanting. Tissue pieces from plants that died during the observation period and all symptomatic plants surviving after 4 weeks were placed into PARP and PARP plus hymexazol media for pathogen isolation.

Pathogenicity testing of fungal isolates from mortality centers

We used several methods to test for pathogenicity of *P. cinnamomi* isolates. Isolates used included 6-7-1A from pear baits in soil from the Apricum Hill mortality center, 6-4-4C from *A. myrtifolia* in the Apricum Hill mortality center, and 6-4-5A from *A. myrtifolia* in the mortality center along SR 88 between Ione Buena Vista Road and SR 104.

AGAR PLUG STEM INOCULATIONS

Agar plug inoculations of wounded stems were conducted using the methods described above with the following modifications. For *A. viscida*, multiple shoots from individual plants were used as replicates for each inoculation treatment. Inoculations of different sets of *A. viscida* plants were performed on 29 May (two replicates per isolate or control), 1 June (two replicates isolate or control) and 9 October (three replicates per isolate or control). One set of *A. myrtifolia* plants was inoculated 9 October (three plants per isolate or control). For May and June inoculations, agar plugs with *cinnamomi* mycelium were cut from margins of young colonies on MEA. For October inoculations, agar plugs with *P. cinnamomi* mycelium were cut from margins of actively growing colonies on V8 juice agar. Parafilm and agar plugs were removed after either 4 days (*A. viscida*) or 5 days (*A. myrtifolia*). For the first 5 days after inoculation, plants were incubated indoors in sunlight at temperatures that ranged from about 20 to 27 C in June and 16 to 22 C in October. For the October inoculations, plants were transferred to the greenhouse (7 to 38 C diurnal temperature range) 5 days after inoculation. We reisolated the pathogen from the margins of cankers of inoculated plants by placing stem pieces (approximately 1-2 mm in diameter) in PARP media.

ZOOSPORE INOCULATIONS OF *A. VISCIDA* AND *A. MYRTIFOLIA* ROOTS

To obtain zoospores of *P. cinnamomi* isolates, we grew colonies on cleared V8 juice agar or cornmeal agar (4% cornmeal, 1.5% agar). The active margin of the colonies were cut into pieces and floated in petri plates containing either 10% nonsterile soil extract or charcoal-filtered tap water for several days to several weeks to allow for sporangium production. The plates containing sporangia were then chilled for 15 minutes at 5 C to induce zoospore release. When zoospore release was maximal, the zoospore suspension was decanted from the plates and zoospore concentrations were determined.

A. viscida plants growing in 473 ml pots were inoculated with zoospores of either isolate 6-4-4C at 3.8×10^5 zoospores/plant or isolate 6-7-1A at 10^5 zoospores/plant on 8 July. Two plants were inoculated for each isolate. Each pot was nested in a larger pot without drain holes and the soil in

the pot was saturated with water immediately before zoospores were added. Additional water was added as needed to flood the soil to the point that a thin film of water was present over the soil surface. Two plants that were flooded in the same manner but to which no zoospores were added served as controls. After flooding for 20 h, plants were allowed to drain and were maintained in a greenhouse with normal to heavy irrigation. All plants were subjected to an additional 24 h flooding treatment 12 days later. Prior to harvesting of the plants 17 days after inoculation, we measured midday SWP as described above. Roots were then examined and isolations were made onto PARP from cankers on the lower stems of inoculated plants.

A. myrtifolia plants grown in 250 ml pots were inoculated with a combination of *P. cinnamomi* isolates 6-4-4C and 6-7-1A on 25 November. Eight plants were inoculated by adding $4.3x10^5$ zoospores to the soil in each pot and flooding for 23 h. Eight control plants were flooded in the same manner but were not inoculated with zoospores. After flooding, the soil was allowed to drain normally. Plants were placed in the greenhouse during the day and inside overnight (diurnal temperature range about 14 to 37 C). Symptom development was observed over the next 18 days at which time the plants were harvested and symptomatic tissue pieces from the roots and crowns of inoculated plants were placed on PARP media.

ZOOSPORE INOCULATION OF *A. VISCIDA* LEAVES

We used zoospores produced for the *A. viscida* soil inoculation to inoculate *A. viscida* leaves on 8 July. We pipetted 2 ml of zoospore suspension into a small plastic bag that was sealed around a leaf. Concentrations of zoospores in the suspensions were $3.1x10^4$ per ml for 6-7-1A and 10^4 per ml for 6-4-4C. Control leaves were exposed in the same manner to non-sterile soil extract. A portion of each bagged leaf remained in contact with the zoospore solution or soil extract for 4 days after which the bags were removed. Plants were kept indoors in a sunny location at temperatures of 20 to 28 C over the course of the experiment. Symptomatic tissue was placed into PARP media 3 weeks after inoculation to reisolate the pathogen.

RESULTS

STEM CANKERS

Disease symptoms in the field

We observed similar symptoms of shoot blighting and branch dieback on *A. myrtifolia* (Figures 3 - 6), *A. viscida* (Figure 7), and *A. manzanita*, but the following descriptions apply specifically to symptoms on *A. myrtifolia*.

Shoots of various sizes showed dieback symptoms in December. The smallest affected shoot tips were 1 - 2 mm or less in diameter. Shoot tip dieback was sometimes associated with old inflorescences (Figure 3). Dieback of larger diameter stems was typically associated with girdling stem cankers that appeared to originate from small blighted shoots (Figures 4, 5). Intercalary stem cankers on stems several mm to a cm or more in diameter were typically elongate and were sometimes sunken (Figure 5). Perennial intercalary cankers on larger stems typically had a narrow (about 1 mm wide) band of discolored tissue at the interface between the dead/decorticated and healthy tissues. In old large cankers on large stems, the center of the canker was often decorticated. Black, rounded fruiting bodies were present at the edges of some cankers. Fruiting bodies that we examined in December 2001 did not contain spores, presumably because they had already been released.

In stems dead for less than a year, the foliage remains attached and is reddish brown. We observed shoots with dull green and newly brown foliage in early June that were not obviously symptomatic in May, suggesting that infected shoots desiccate as temperatures and evaporative demand increase in late spring. As dead shoots age, the dead foliage becomes darker and/or more gray or black and eventually begins to fall off. The oldest affected stems are defoliated. Data from individual cankers observed between March and October indicates that leaves on branches killed in the late winter or spring generally remain light brown over the summer. The leaves become dull or gray over the rainy season. Most branches with old dead leaves in March (which had died the previous year) were defoliated by October. Hence, the time span from the onset of visible foliar necrosis to defoliation is typically at least 1 to 1.5 years.

In some locations, including a site off Lambert Road, we observed individual plants that appeared to have been killed by incremental expansion of stem cankers (Figure 6). Plants in these locations were apparently growing on very poor soil: Stands were sparse and plants were extremely short. Dead plants had numerous bleached dead branches which had apparently accumulated over a number of years. In some cases, rooted stems on the plant periphery appeared to be surviving the death of the plant's main stems. The bases of the larger stems of these declining and dead plants typically had extensive boring by beetles (possibly buprestid) that may contribute to their final collapse. Although some localized areas showed a relatively high incidence of this type of decline, affected plants were scattered and did not occur in discrete patches. This pattern of mortality was distinct from the pattern of mortality associated with *P. cinnamomi* discussed below.

Figure 3. Dieback of shoot tips associated with stem canker fungi on *A. myrtifolia* (December 2001). Cankers that involve cause the dieback of individual shoot tips are referred to as terminal cankers.

Figure 4. Recent branch dieback (top) and associated stem canker (detail, bottom) in *A. myrtifolia.* Bark at base of dead stem shown in top photo has been sliced away to reveal the interface between the brown necrotic canker and healthy green tissue (January 2003).

Figure 5. Stem cankers on *A. myrtifolia* (December 2001). Note junction between live and dead tissue in stem in upper photograph where bark has been sliced away. Lower photograph shows a persistent intercalary canker on large stem.

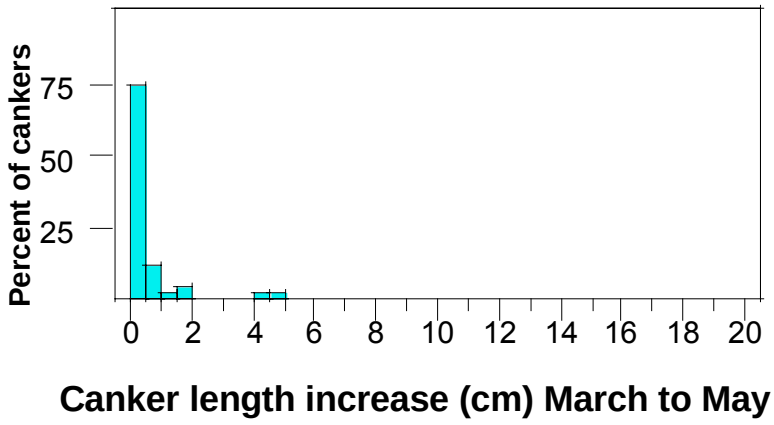
Figure 6. Declining and dead *A. myrtifolia* possibly killed by stem canker fungi (December 2001).

Figure 7. Shoot dieback on *A. viscida* associated with stem canker fungi at Apricum Hill (December 2001).

Stem canker expansion

Many of the individual stem cankers that we monitored in field plots between March and October did not expand visibly (Figure 8). Half (8/16) of the intercalary cankers and 36% (8/22) of the

terminal cankers did not increase in length over the observation interval. Although most increases in canker length were small, several cankers expanded substantially (Figure 8). In one instance, an entire branch that included a monitored terminal canker died between February and May due to expansion of another non-monitored canker farther down the stem. Average canker growth rates did not differ significantly between terminal and intercalary cankers and canker size in March was not significantly correlated with the amount of canker growth. Observed amounts of canker elongation did not differ between plants in the upper and lower plots.



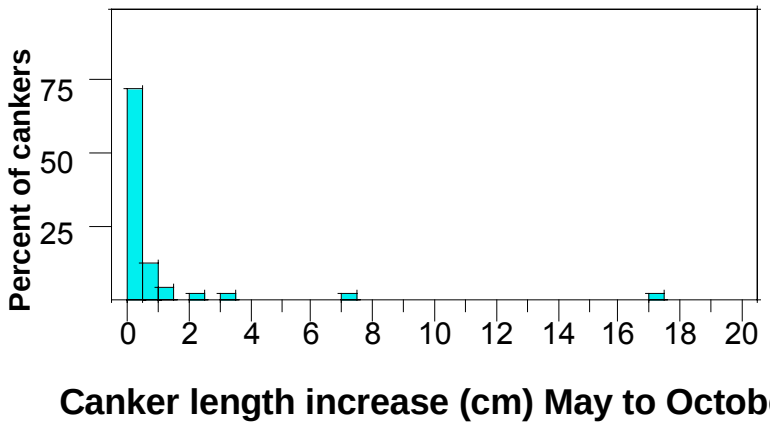


Figure 8. Histograms of canker length increases from March to May and May to October 2002 (n=40).

Overall disease levels in plots

Plants in the upper plot at Apricum Hill generally appeared healthier and less stressed than those in the lower plot (Figure 2). In March, only four of the 36 tagged plants in the upper plot showed recent dieback compared to 19 plants in the lower plot. The average rating for recent dieback in the upper plot (mean = 0.1) was significantly less (t-test $p = 0.0005$) than in the lower plot (mean = 0.5). Ratings for defoliated stems and total dieback showed the same trend, but mean rating differences were only significant at $p < 0.10$.

Transect ratings of overall plot cover are shown in Figure 9. Overall, the lower plot had significantly less healthy foliage (likelihood ratio $p = 0.002$) and more bare ground (likelihood ratio $p = 0.011$) than the upper plot. Cover ratings did not change significantly between February and October.

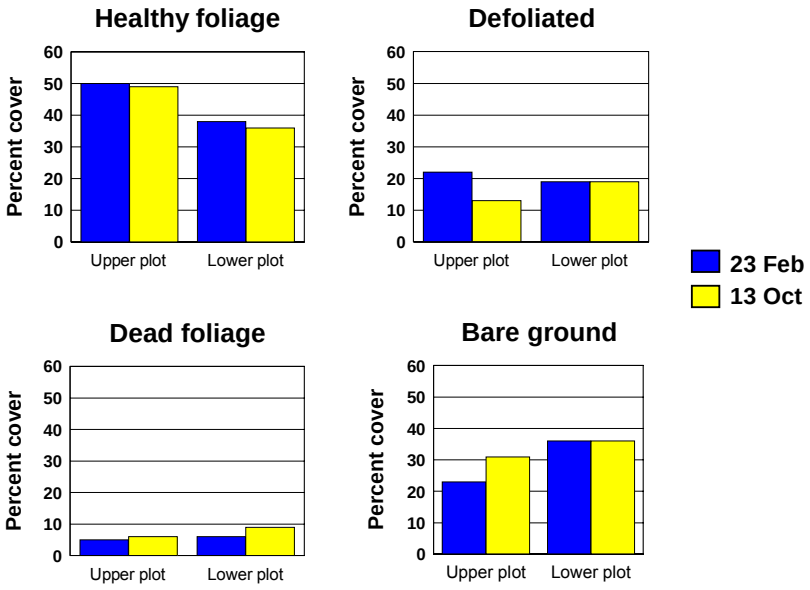


Figure 9. Cover ratings in the upper and lower plots at Apricum Hill in February and October 2002.

Water stress in field plots

Stem water potential (SWP) readings (Figure 10) show that plants in the lower plot developed greater levels of water stress than those in the upper plot. In both May and October, SWP values in the upper plot were significantly higher (i.e., less negative) than those in the lower plot (May t-test $p = 0.036$; October, t-test $p < 0.0001$ for hypothesis that upper plot SWP is greater than -4 MPa). The high levels of water stress experienced by plants in the lower plot probably contributes to the reduced live plant cover observed in the lower plot relative to the upper plot (Figure 9).

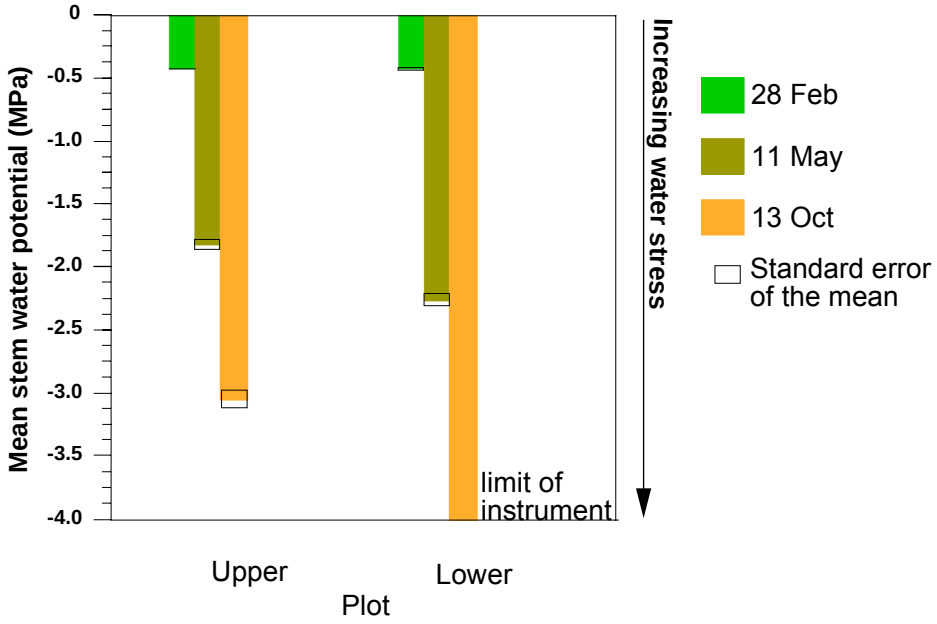


Figure 10. Average midday stem water potentials (MPa) of *A. myrtifolia* in the upper and lower plots at Apricum Hill on three dates in 2002. Observed declines in SWP are related to declining soil moisture levels as the season progresses. The permanent wilting point for most agronomic plants is -1.5 MPa. The pressure chamber used was not capable of measuring water potentials less than -4 MPa.

Growth in field plots

Tagged plants in the upper plot averaged 40 cm in height compared to 26 cm in the lower plot (t-test significant at $p < 0.0001$). Individual plant height showed a significant positive correlation with May SWP values (Figure 11). Plants that experienced high levels of water stress by May were shorter than plants that were less stressed by May. Average shoot growth over the period March to October did not differ significantly between plots (average growth 0.73 cm in lower plot compared to 1.2 cm in upper plot). Differences between the plots may have been minimized because our observation period did not include the wet winter months, a period of active growth during which time *A. myrtifolia* plants flower (December-January) and set seed. Also, a number of shoots failed to grow over the summer because their tips had died. Ten of 30 shoots in the lower plot and 5 of 30 shoots in the upper plot that were used for measurements had died back at least partially by October.

The direction of the differences in overall plot averages for SWP, plant height, dieback related to stem cankers, and healthy plant cover suggest that these factors may be correlated. Although individual plant height showed a significant positive correlation with May SWP (Figure 11), we did not observe significant relationships between the amount of canker elongation and either shoot growth or SWP over the observation period (March to October). Changes in canker size and shoot length during the study period were small and variation within and between plants was considerable. We believe that a longer monitoring period and/or larger sample size would be needed to reliably determine whether canker expansion is related to SWP or growth rate on an individual plant basis.

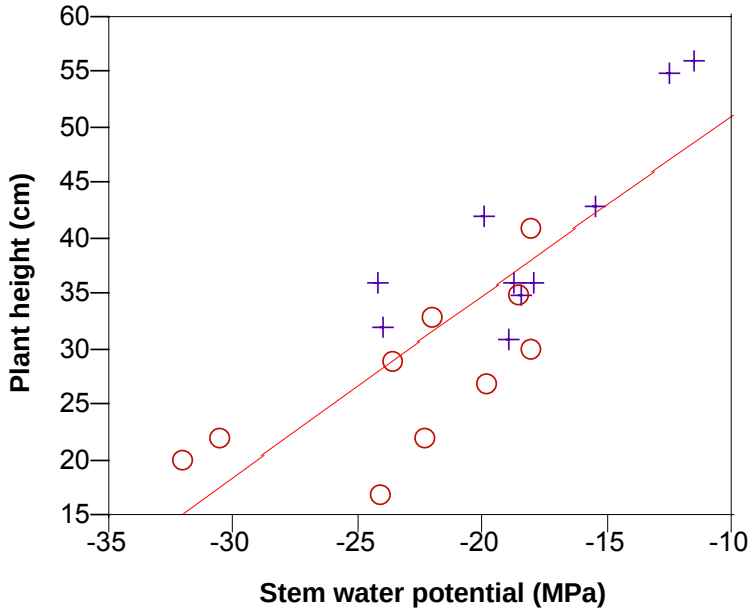


Figure 11. Relationship between February plant height (cm) and May midday stem water potential for *A. myrtifolia* in the upper (+) and lower (○) plots at Apricum Hill. Plotted regression line is significant at $p<0.0001$ (adjusted $R^2=0.639$).

Stem canker isolations

Isolations from stem cankers sampled on 15 December 2001 yielded a number of different fungi on aPDA and MEA but no fungi grew from pieces plated on PARP (semi-selective for *Phytophthora*

spp.). After discarding likely saphrophytic fungi, we selected three types of colonies for pathogenicity testing.

Fungal isolate G and a number of morphologically identical isolates were obtained on both MEA and aPDA from large *A. myrtifolia* stem cankers collected from Apricum Hill and the Lambert Road area. This fungus also was isolated from leaf spots on *A. manzanita* from the Lambert Road area. It produced floccose white colonies which became gray in the center (Figure 12). Spores produced in pycnidia formed in culture on PDA were fusiform and measured on average 30.4 µm long by 6.9 µm wide. The fungus was keyed out to the genus *Fusicoccum*.

Isolate J was fast growing with initially white fluffy aerial mycelium which quickly became gray (Figure 12). It was isolated from a canker on an *A. viscida* twig collected from the Lambert Road area. This fungus formed pycnidial initials after 1 week on aPDA, but no spores were ever observed within the pycnidial initials in culture.

We also tested isolate H, which produced black, slimy, slow-growing colonies on aPDA. It was isolated from twigs and leaves of *A. myrtifolia* and *A. viscida* in both the Lambert Road and Apricum Hill areas.

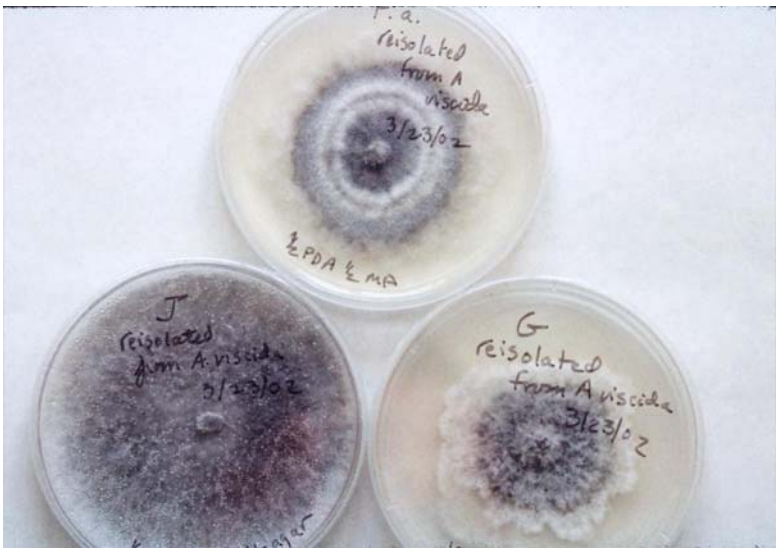


Figure 12. Transfers of cultures (6 days old) reisolated from *A. viscida* plants inoculated with agar plugs growing on PDA-MEA (1:1) agar. Top: *Fusicoccum aesculi* obtained from CDFA (originally isolated from *Sequoia sempervirens*). Lower left: Isolate J, a fast-growing *Fusicoccum* sp. originally isolated from *A. viscida* stem canker. Lower right: Isolate G, *Fusicoccum* sp. (possibly *F. aesculi*) originally isolated from *A. myrtifolia* stem cankers.

Pathogenicity testing of fungi associated with stem cankers

In February, we inoculated *A. viscida* plants with agar plugs cut from cultures of isolates G, J, and H and an isolate identified as *Fusicoccum aesculi* obtained from Tim Tidwell of CDFA (originally from *Sequoia sempervirens* growing in Elk Grove, CA). Only isolates G and J, which were pathogenic in this initial pathogenicity test, were used in a second round of agar plug inoculations of both *A. viscida* and *A. myrtifolia* in October.

FEBRUARY INOCULATIONS OF *A. VISCIDA*

By 2 weeks after inoculation with sterile agar plugs, control wounds on *A. viscida* turned brown and discoloration extended 1 to 2 mm around the initial wound point. Plants inoculated with *F. aesculi* (CDFA isolate) and isolate H remained similar in appearance to the control. No growth occurred from any of the isolations made from the control inoculations, but the CDFA *F. aesculi* was successfully reisolated from plants inoculated with this fungus.

By 2 weeks after inoculation with isolate G (originally from *A. myrtifolia*), discoloration extended about half the circumference of inoculated stems. In contrast, for isolate J (originally from *A. viscida*) discoloration extended about 80% of the stem circumference and shoot tips had begun to desiccate. By 4 weeks after inoculation, discoloration associated with isolates G and J had completely girdled the inoculated stems, killing all but one (from isolate G) of the shoot tips distal to the inoculation points. Cankers from both isolates were 2 to 2.5 cm long after 4 weeks. We reisolated G and J from stem cankers of their respective inoculated plants (Figure 12).

OCTOBER INOCULATIONS OF *A. VISCIDA* AND *A. MYRTIFOLIA*

***A. viscida*.** On *A. viscida*, disease development from agar plug inoculations was more rapid in October than in February. Also, isolate G showed greater virulence and isolate J showed less virulence in the October inoculations than had been the case in the February inoculations (Figure 13). Control plants inoculated with sterile agar plugs did not develop symptoms and no fungi were isolated from the wounds of control plants.

By 19 days after inoculation with isolate G, cankers ranged in length from 4 to 9 cm and all shoot tips distal to the inoculation points had been killed. By 4 weeks after inoculation, numerous fruiting bodies (pycnidia) were observed on the killed stems (Figure 14). Pycnidia had not been observed on plants inoculated in February. Under microscopic examination, pycnidia released copious amounts of spores when wetted with water. Pycnidia and spores (Figure 15) were keyed to the genus *Fusicoccum*. Isolate G spores formed on *A. viscida* averaged 28.1 μm in length and 7.6 μm in width, with an average length to width ratio of 3.7. Isolate G was reisolated from cankers on inoculated shoots.

Cankers produced by isolate J at 19 days averaged about 1 cm long and did not girdle stems (Figure 13). By 2 months after inoculation, cankers averaged about 1.5 cm long and 2 of 3 inoculated stems were completely girdled. Isolate J cankers did not produce fruiting bodies within 4 weeks, but minute pycnidia were found on cankered areas after 2 months (Figure 15). These were the only spores we observed for this isolate because spores have not been produced in culture to date. Pycnidia were smaller and much sparser than those formed by isolate G. Spores were similar in appearance to isolate G but smaller. Isolate J spores averaged 23 μm long and 7.1 μm wide, with an average length to width ratio of 3.3. Isolate J was also keyed to the genus *Fusicoccum*, and most likely represents a different species than isolate G. Isolate J was reisolated from the cankers on inoculated shoots.

Figure 13. *A. viscida* inoculated with isolate G (left) and isolate J (right) 2 weeks after inoculation in October. The three shoot tips inoculated with *Fusicoccum* isolate G are dead.

Figure 14. Close-up of fruiting bodies of *Fusicoccum* sp. isolate G along dead *A. viscida* stem one month after the October inoculation.

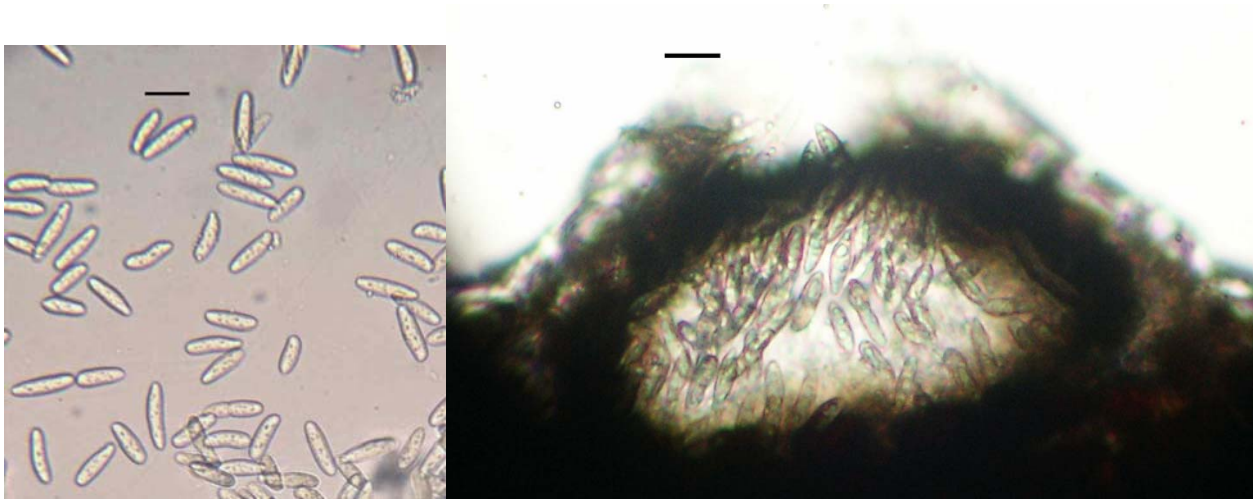


Figure 15. At left, spores of *Fusicoccum* sp. isolate G from fruiting bodies on *A. viscida*. At right, freehand cross section through *A. viscida* stem, showing erumpent subepidemal pycnidium and spores of *Fusicoccum* sp. isolate J. Scale bars are approximately 20 µm.

A. myrtifolia. Isolates G and J were also pathogenic to *A. myrtifolia* (Figures 16 and 17). Within 5 days of inoculation, necrotic reactions were evident at inoculation points. On two of three plants inoculated with isolate G, cankers expanded rapidly, girdling the stems and killing the plants within 11 days of inoculation. On the third plant, the canker remained about 5 mm long a month after inoculation and did not girdle the stem. Isolate G was reisolated from lesions of all three plants. We were unable to observe any fruiting bodies on killed stems, which may be related to the very small stem diameters of these plants (about 0.5 mm).

Cankers produced by isolate J expanded more slowly than those caused by isolate G. Girdling cankers about 25 mm long developed on two of three inoculated plants after about 2 weeks, killing them, while the canker on the third plant was about 2 mm long and did not girdle the stem. Isolate J was reisolated from all three plants.

Wounds of the three shoots on the control *A. myrtifolia* plant inoculated with sterile agar plugs developed brown callus, but remained healthy.

Figure 16. Cankers caused by isolate G on *A. myrtifolia* 14 days after inoculation.

Figure 17. Left: *A. myrtifolia* dying as result of agar plug inoculation with *Fusicoccum* isolate J 14 days after inoculation. Dead leaves of this plant are dull green. Right: Control plant 6 weeks after inoculation; note new growth.

ROOT DISEASE CENTERS

Disease symptoms in the field

The mortality center at the Apricum Hill Preserve that we first observed in March 2002 covers about 0.25 ha (Figure 18). Because it includes at least one of the rebar-marked 1 x 2 m permanent plots established by Wood and Parker (1989) but was not mentioned in their report, it clearly postdates their surveys. Based on the condition of the dead and defoliated plants, it appears that most of the mortality in this patch occurred at least 2 years before our observations were made.

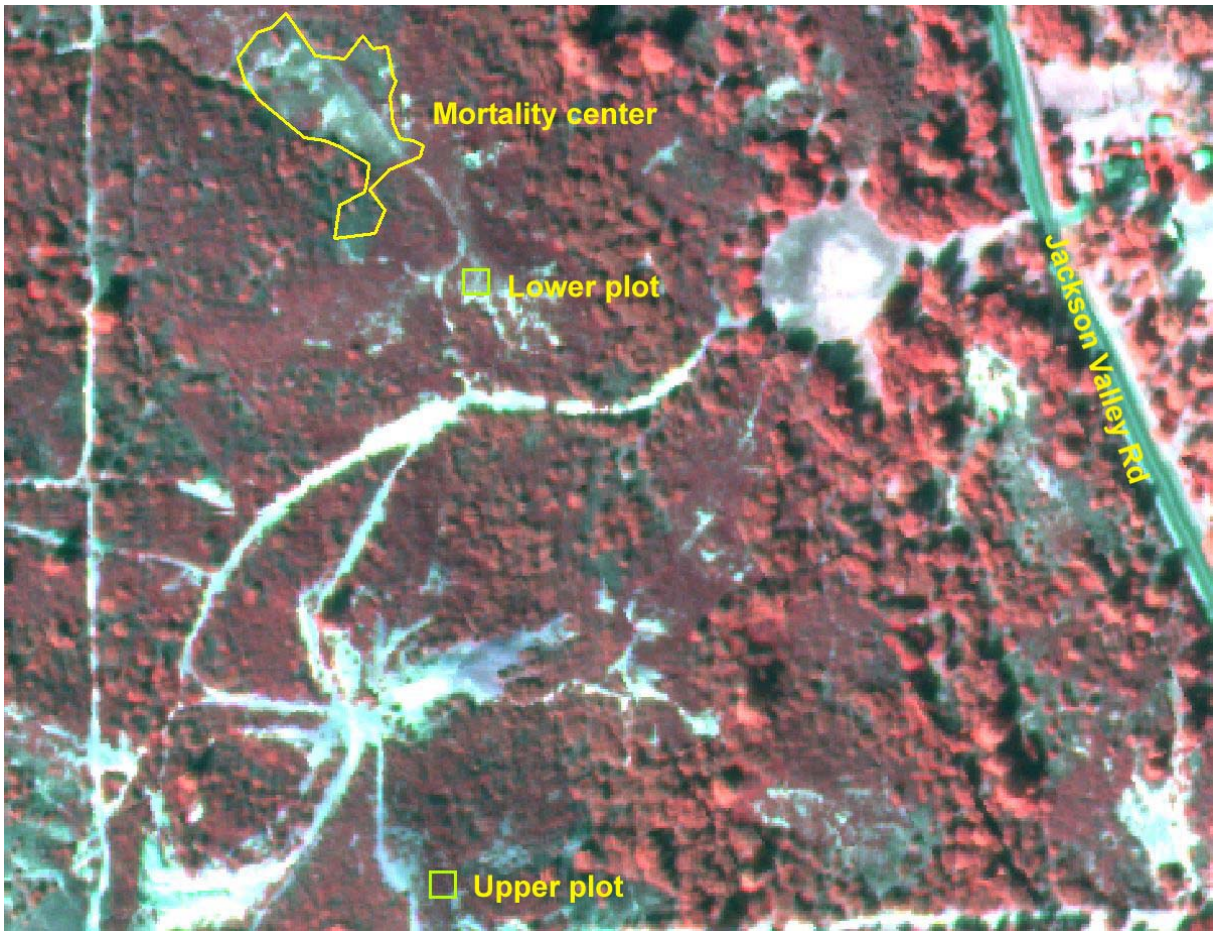


Figure 18. Part of the CDFG Apricum Hill Preserve showing the outline of mortality center in March 2002 (from GPS coordinates) and locations of the lower and upper plots (squares, about 10 m x 10 m). Aerial imagery from California Department of Forestry and Fire Protection AIRIS imagery August 2002, courtesy Tiffany Meyers.

Almost all *A. myrtifolia* and *A. viscida* plants within the mortality center were dead, and most showed similar levels of weathering of the dead stems, indicating that many plants died within a relatively short time period (Figure 19). Some clusters of asymptomatic plants were present within the affected area (Figures 19, 20). Most of these were located on strips of soil that had a different surface soil and/or were slightly elevated relative to adjacent affected areas. The mortality center had a very abrupt border: dead and apparently asymptomatic plants were adjacent to each other along much of the edge of the affected area.

Very few declining or recently-killed plants were visible within the mortality center in March. However, some of the remaining live plants within the mortality center died between early May

and early June (Figure 20). Root systems of most dying or recently-killed plants had distinct dark brown necrosis of the taproot which sometimes extended up to the root crown and/or into other large roots. Fine roots were sparse, being limited to areas such as the root crown (when healthy) or along the major roots (Figure 21). Fine root density in adjacent healthy areas was substantially greater than in affected areas.

A number of mortality centers similar in appearance to the one at Apricum Hill are located within 2 km of the preserve and can be seen from the hill on the preserve. Many of these are adjacent to roadsides and show a spreading pattern typical of soil-borne root diseases. George Hartwell (personal communication) indicated that most of the mortality centers visible in the area had developed and/or expanded over the previous 5 years or so.

One of the largest mortality centers, located along SR 88 between Ione Buena Vista Road and SR 104, is specifically mentioned in Wood and Parker's (1989) report (Figure 22). Recently killed patches of surviving *A. myrtifolia* and *A. viscida* are found near the periphery of the affected area, but only highly deteriorated plant remains were left in the middle of the mortality center due to the amount of time that had elapsed since the plants had died. Like the Apricum Hill mortality center, this area contained some patches of surviving *A. myrtifolia* and *A. viscida*, mostly on upper slopes and ridges. We observed some regeneration of both species near the edges of some surviving patches, but we also saw young plant of *A. myrtifolia* and *A. viscida* that were dying or had been killed recently (Figure 23). Some of the affected plants had only recently wilted in June (Figure 23). Affected plants showed dark brown discoloration of the taproot and root crown and evidence of root decay of fine roots.

Symptoms of disease in excavated root systems were most obvious in June, when recently-killed plants were present. Earlier in the season (February through early May) affected plants did not show top symptoms. Root rot symptoms were also difficult to distinguish late in the season after killed plants had dried out.

Although both *A. myrtifolia* and *A. viscida* were killed in mortality centers, other species were apparently unaffected (Figure 22). Apparently healthy regeneration of *Quercus wislizeni* and *P. sabinianum* were present within mortality centers, although some dead *Q. wislizeni* seedlings were seen. We also observed larger *Q. wislizeni* and *Q. berberidifolia* plants within the mortality centers that did not exhibit top symptoms indicative of root decay.

Figure 19. Views of the root disease center at Apricum Hill Preserve taken from a point near the southeast corner of the affected area. Top: Dead *A. myrtifolia*, view west southwest, February 2002. Bottom: Edge of root disease center, view southeast, May 2002. Note narrow band of live plants between 2 dead areas (left center).

Figure 20. Recent mortality in previously unaffected island of plants in the mortality center at Apricum Hill Preserve. View from the western edge looking east, October 2002. Note dead *A. myrtifolia* plants in foreground which have not yet lost their leaves and dead defoliated plants in background.

Figure 21. Excavated root crown of recently-killed *A. myrtifolia* in the Apricum Hill mortality center, June 2002.

Figure 22 (previous page). Extensive old mortality center on the north side of SR 88 between Ione Buena Vista Road and SR 104 in June 2002 (top) and January 2003 (center and bottom). Dead *A. myrtifolia* have mostly decayed and are no longer apparent in portions of the top and center images. Bands of live *A. myrtifolia* in all three photos are on ridges. Large dark green plants recruiting in opening formed by *A. myrtifolia* mortality (top and center photos) are primarily *Q. wislizeni.* Also note tracks created by vehicles (top, center) that may have helped move infested soil upslope and disseminate the pathogen throughout the area.

Figure 23. Dying young *A. myrtifolia* (top) and *A. viscida* (bottom) in large mortality center along SR 88. *A. viscida* are in portion of mortality center south of SR 88. Note dead mature *A. viscida* plants in the background (bottom).

We established three point-intercept transects in the Apricum Hill mortality center that spanned areas with live and dead plants in order to track expansion of the mortality center. Transects were assessed in March, May, and October. We observed little change in the extent of the mortality center between March and October. The only meaningful change seen over this period was the appearance of recently-killed *A. myrtifolia* and *A. viscida* foliage within two of the transects (Figure 24). Recent mortality along the transects was first observed in May. Degradation of old dead plants may also have contributed to the slight increase in bare ground in the October ratings (Figure 24).

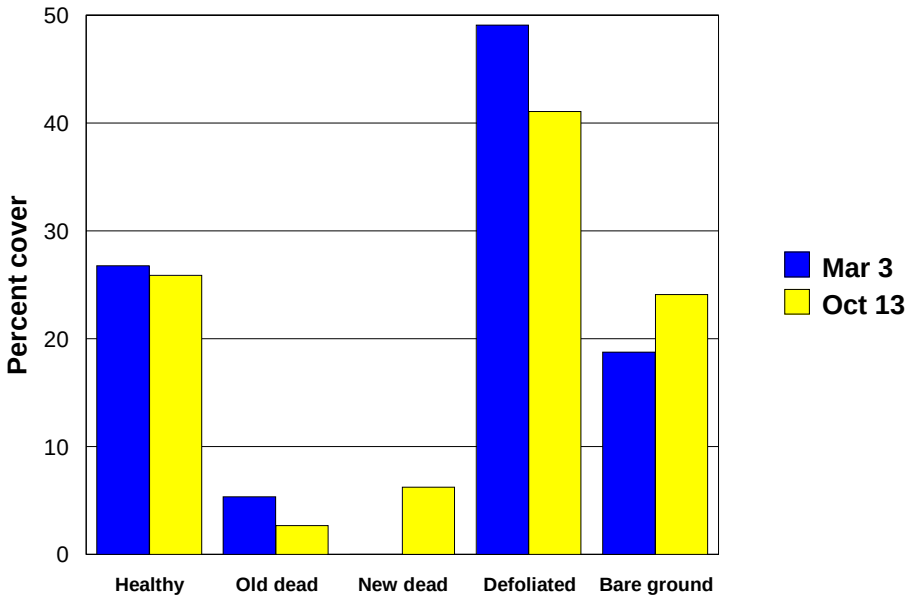


Figure 24. Average cover ratings for *A. myrtifolia* and *A. viscida* (combined data) by symptoms class in March and October 2002 for three transects located in the mortality center at Apricum Hill (n=112 sample points).

Isolation and identification of *Phytophthora cinnamomi*

SOIL BAITING

Because field symptoms were consistent with a root disease and suitable plant material for isolations was not available initially, we collected soil to test for the presence of *Phytophthora* species by baiting with green pears. Positive baiting results were obtained from two different soil samples collected from the Apricum Hill mortality center in May. Pears exposed to mortality center soil developed numerous brown lesions by the end of the 48 h soaking period, and the lesions expanded significantly within the following 18 h (Figure 25). Isolations from lesions on pear baits mainly yielded an oomycete that produced mycelium with numerous hyphal swellings that was subsequently identified as *P. cinnamomi* (Figure 26, discussed below). A second unidentified fungus was isolated from one tissue piece.

Green pears are only semi-selective for *Phytophthora* species. Some members of the related genus *Pythium* can also infect green pears (especially when incubated at high temperatures) and other fungi can also infect pears through small wounds. Nonetheless, pears incubated in soil collected from a healthy stand of *A. myrtifolia* at the Apricum Hill preserve remained free of symptoms (Figure 25).

The first round of baiting was conducted at an average temperature of about 25 C. We ran a second set of pear baiting trials at cooler flooding temperatures (average about 15 and 17 C for the two trials, respectively) to see if pathogens with lower temperature optima were present. In these cool temperature trials, pears placed in the two soil samples from the Apricum Hill mortality center had only a few brown spots when removed from the soil solutions. These brown spots enlarged rapidly after several days at room temperature (25 C). Most of the spots appeared to be wound infections, and some were on the bottom of the fruit rather than along the water line. Isolations from spots consistently yielded *P. cinnamomi*. No other fungi were isolated and no spots developed on pears incubated in the soil sample from the healthy area.

Figure 25. Pear baits 18 h after removal from flooded soil samples collected from Apricum Hill. Each pear was placed in contact with a separate soil subsample. Pears with brown lesions at left and center were incubated with samples from the mortality center (Apricum Hill samples 1 and 2, respectively, Table 2). The two asymptomatic pears at the right were incubated with soil collected from an unaffected area (Apricum Hill sample 3).

We tested the pH and electrical conductivity of saturation paste extracts of the three soil samples to determine whether soil properties differed between samples from the mortality center and the healthy area. As shown in Table 2, all three samples had similarly low pH and conductivity values.

Table 2. Soil samples collected from Apricum Hill

Sample	Disease status	Locality description	pH	EC (mS/cm)
Apricum Hill 1	Diseased	single sample near a severely declining *A. viscida* within about 10 m of the edge of the mortality center	4.2	0.12
Apricum Hill 2	Diseased	bulked sample from near various dead plants in central portion of mortality center	4.5	0.10
Apricum Hill 3	Healthy	bulked sample from near several healthy plants in a nonaffected area about about 50 m south of the mortality center	4.4	0.05

PLANT ISOLATIONS

With only limited sampling in March, we did not find very suitable plant material for isolations, and initial isolations from roots were unsuccessful. On 3 June we sampled more widely and were able to find plant material showing clear root rot symptoms. We isolated *P. cinnamomi* on PARP,

PARP with hymexazol, and aPDA media with varying efficiencies from symptomatic plants collected from all six areas that we sampled. The pathogen was recovered from *A. myrtifolia* sampled in three sites within the Apricum Hill mortality center and from both *A. myrtifolia* and *A. viscida* sampled from 3 locations in the SR88 mortality center (2 sites north and one site south of the highway). Isolates 6-4-4C and 6-4-5A, from roots of *A. myrtifolia* at Apricum Hill and the north side of SR88, respectively, were used in various studies discussed below.

PATHOGEN IDENTIFICATION

Our isolates from soil and plant material did not readily form sporangia in culture, so we were unsure initially whether they represented a species of *Phytophthora* or *Pythium*. With the assistance of Dr. Greg Browne (Dept. of Plant Pathology, UC Davis), we set up a small trial to induce sporangium production at 3 temperatures (14 C, 18 C, and 21 C) in agar plugs cut from 5 day old cultures grown on either cleared or standard V8 juice agar and incubated in nonsterile soil extract. After 2 days, abundant sporangia were formed on cleared V8 juice agar plugs held at 21 C and very few were present on cleared V8 juice agar at 18 C. No other treatment produced sporangia by that time. By observing sporangia and zoospore release, we were able to identify the isolates as a *Phytophthora* species.

All *Phytophthora* isolates exhibited virtually identical morphological characteristics. They produce numerous chlamydospores in culture and hyphae are gnarled in appearance due to the presence of hyphal swellings (Figure 26). Sporangia are ellipsoid, and nonpapillate, averaging about 62 x 38 μm (L:W ratio 1.6) with an exit pore diameter of about 10 μm. Zoospores are about 10 x 15 μm. These characteristics and other characteristics, including the observed temperature range for sporangium production, are consistent with the description of *P. cinnamomi* Rands, a well-known and widely distributed root pathogen with a very wide host range.

We sent culture 6-4-4C (from *A. myrtifolia* roots, Apricum Hill) to Dr. Matteo Garbelotto, (Forest Pathology and Mycology, Department of Environmental Science, Policy, and Management, Ecosystem Sciences Division, UC Berkeley) for identification using molecular methods. In his lab, DNA was extracted from the mycelium, amplified via polymerase chain reaction (PCR) using primers ITS1 and ITS4, and the sequence of the internal transcribed spacer (ITS) of the nuclear ribosomal DNA was determined. By comparing this sequence to published sequences for *Phytophthora* spp. in GenBank, Dr. Garbelotto was able to confirm that the isolate is *P. cinnamomi*.

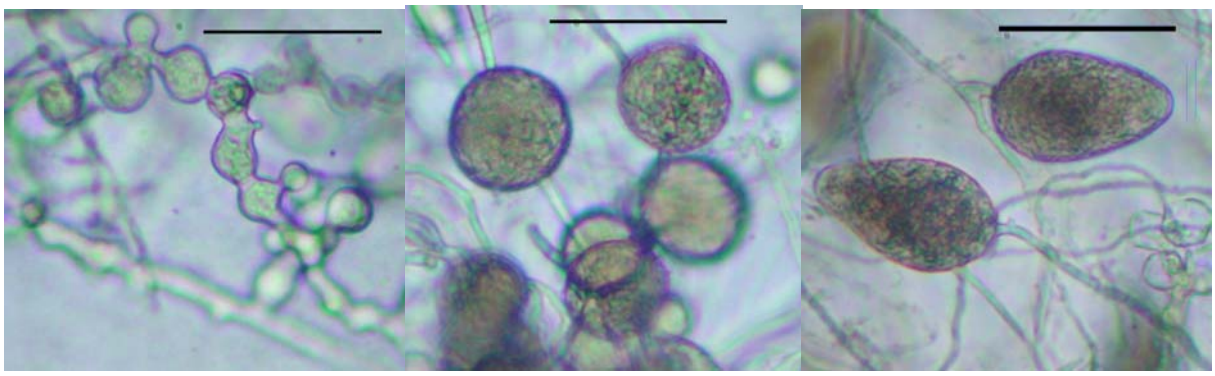


Figure 26. Light micrographs of *P. cinnamomi* isolated from *A. myrtifolia.* Mycelium with hyphal swellings (left), chlamydospores (center), and sporangia (right). Scale bars are approximately 60 µm.

Pathogenicity testing of *P. cinnamomi* on *A. viscida* and *A. myrtifolia*

AGAR PLUG INOCULATIONS

In May we screened the two isolates types from green pears for pathogenicity to *A. viscida* stems with agar plugs. By three days after inoculation, the shoot tip of the plant inoculated with isolate 6-7-1A (subsequently identified as *P. cinnamomi)* was completely wilted and discoloration had extended through the vascular tissue into the leaves (Figure 27). *P. cinnamomi* was readily reisolated from symptomatic stem tissue. No discoloration or wilting was evident in plants inoculated with the other unidentified isolate or with sterile agar plugs.

This experiment was repeated in June using only isolate 6-7-1A (two plants plus control) with similar results. By 6 days after inoculation, discoloration extended up to 5 cm beyond the inoculation point. We readily reisolated *P. cinnamomi* from these cankers. We obtained similar results when agar plug inoculations of *A. viscida* with *P. cinnamomi* were repeated in October.

Figure 27. Shoot and leaf discoloration of *A. viscida* 3 days after inoculation with *P. cinnamomi* isolate 6-7-1A recovered from pear baits. The agar plug used to inoculate the shoot has dehydrated and is adhered to the stem at the inoculation point (center of photo).

We inoculated shoots of rooted *A. myrtifolia* cuttings using agar plugs of two *P. cinnamomi* isolates (6-4-4C from *A. myrtifolia* at Apricum Hill, and 6-4-5A from *A. myrtifolia* along Hwy 88). Five days after inoculation, we observed dark discoloration of the stem near the inoculation point in all six inoculated plants, whereas no stem reaction was observed on the control plants. By two weeks after inoculation, all of the plants inoculated with *P. cinnamomi* were dead (Figure 28), while the controls remained healthy. *P. cinnamomi* was reisolated from two of three plants inoculated with isolate 6-4-5A and all three plants inoculated with isolate 6-4-4C.

Figure 28. *A. myrtifolia* plant killed by agar plug inoculation with *P. cinnamomi*, 14 days after inoculation.

INOCULATION OF *A. VISCIDA* AND *A. MYRTIFOLIA* WITH *P. CINNAMOMI* ZOOSPORES

Root disease caused by *P. cinnamomi* is typically initiated by zoospores, motile spores that are released from sporangia when soils are saturated. We added *P. cinnamomi* zoospores to the soil of pots containing plants of *A. viscida* or *A. myrtifolia* to test the ability of our isolates to initiate disease via zoospores.

Three of four *A. viscida* plants inoculated with *P. cinnamomi* zoospores developed basal stem cankers by 17 days after inoculation. Among the two plants inoculated with isolate 6-7-1A, one developed a canker 10 cm long and the other a less well-defined canker about 6 cm long. One stem canker about 5 cm long developed on one of the two plants inoculated with 6-4-4C. No crown cankers developed on control plants.

Mature leaves of *A. viscida* are stiff and resistant to wilting, so top symptoms on plants with even severe root and crown rot can take a long time to develop. Because we wanted to attempt to reisolate the pathogen from inoculated plants, we did not allow affected stems to completely dry up. We measured midday SWP of the plants just before the experiment was terminated to determine the amount of water stress associated with root rot (Figure 29). The noninoculated control plants had the highest (least negative) SWP readings, indicating that these plants were the least water stressed. All four plants inoculated with *P. cinnamomi* had much lower water potentials than the controls and plants with stem cankers had the lowest readings overall. Since all plants received similar irrigation, SWP readings indicated that inoculated plants were desiccating due to root disease and/or loss of vascular tissue in the lower stem.

When the *A. viscida* root systems were rinsed out, the plants inoculated with *P. cinnamomi* exhibited decay of both large and fine roots, which were nearly black. No new root growth was seen in the inoculated plants. In contrast, roots of control plants were more brown than black overall, and had new roots that were light brown to whitish. *P. cinnamomi* was reisolated from stem cankers of the two plants inoculated with isolate 6-7-1A, but was not reisolated from the single stem canker that developed on one of the plants inoculated with isolate 6-4-4C.

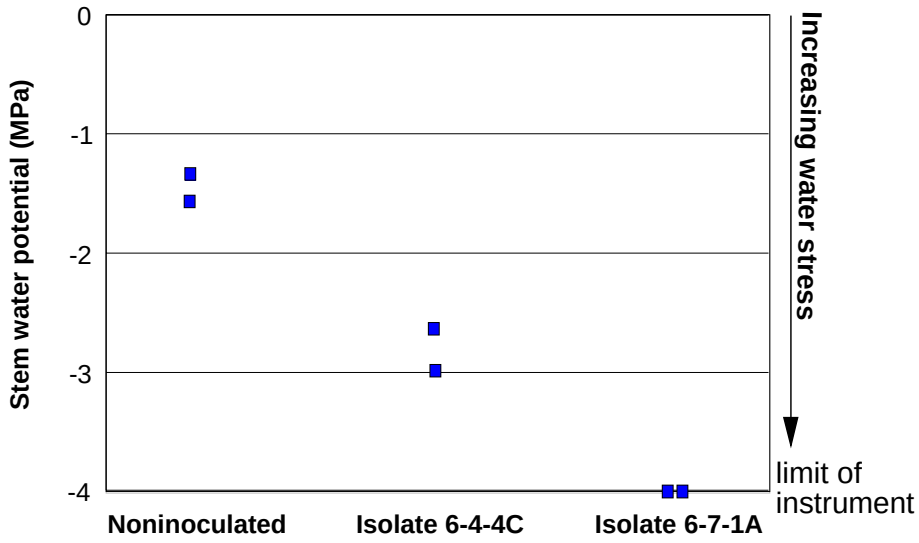


Figure 29. Individual midday stem water potentials (MPa) of potted *A. viscida* in response to infection of *P. cinnamomi* (two plants per treatment) measured 25 July, 17 days after inoculation. The pressure chamber used was not capable of measuring water potentials less than -4 MPa.

We used a mixture of zoospores from isolates 6-4-4C and 6-7-1A to inoculate *A. myrtifolia* plants because total zoospore production for this experiment was somewhat low. By 3 days after inoculation, we observed wilting of young shoot tips in half of *A. myrtifolia* plants to which *P. cinnamomi* zoospores had been added. Control plants showed no such wilting. Affected plants did not recover from wilting and additional inoculated plants exhibited progressively greater wilting symptoms over time. By 17 days after inoculation, all inoculated plants were wilted and 7 of 8 inoculated plants had become dry and discolored, whereas control plants remained green and turgid (Figure 30). Roots systems of inoculated plants were black and decayed and plants showed discoloration of internal stem tissues (Figure 31). We reisolated *P. cinnamomi* from the roots and/or lower stems of all inoculated plants.

Figure 30. *A. myrtifolia* plants 17 days after inoculation of soil with zoospores of *P. cinnamomi* (bottom row) and control plants that were not inoculated (top row).

Figure 31. Roots and stem of *A. myrtifolia* 17 days after inoculation with *P. cinnamomi* zoospores (right) compared with noninoculated control (left) The outer bark at the base of both stems has been sliced away to reveal internal tissues. White material on roots is perlite.

ZOOSPORE INOCULATION OF *A. VISCIDA* LEAVES

We also tested the ability of *P. cinnamomi* spores to infect nonwounded leaves of *A. viscida*. Within 4 days, leaves inoculated with zoospores developed dark necrotic spots and lesions, mainly along leaf margins. Lesion development was greater in the leaf inoculated with the higher number of zoospores. Lesion size ranged from small spots (about 0.1 mm) to areas about 5 by 10 mm. The lesions continued to enlarge and coalesce over the first 24 h after the leaves were removed from the plastic bags. Subsequently, plants were maintained at about 50% relative humidity and we observed no further growth in lesion size over the next 2 weeks (Figure 32). No lesions developed on control leaves exposed to nonsterile soil extract only. Two weeks after inoculation, *P. cinnamomi* was reisolated from spots on both inoculated leaves.

Figure 32. Symptoms of *P. cinnamomi* infection on *A. viscida* leaf (with white tag) two weeks after inoculation with zoospores of isolate 6-4-4C.

A. VISCIDA AND *A. MYRTIFOLIA* TRANSPLANTED INTO NATURALLY INFESTED FIELD SOIL

Transplanting susceptible hosts into soil collected from symptomatic and asymptomatic areas allowed us to test how *P. cinnamomi* functions in the unique soils in which *A. myrtifolia* grows. It also indicates whether inoculum levels present in the soil are sufficient to initiate disease under a given set of conditions.

A. viscida. Our initial test using *A. viscida* was initiated before we had any information on the nature of the pathogen, and so was probably not optimized for expressing *P. cinnamomi* pathogenicity. We imposed only one fairly short (13 h) flooding period 6 weeks after plants were transplanted and 8 days before the experiment was terminated.

One plant died within a month of being transplanted into soil sample 2 (Table 2) from the mortality center at Apricum Hill (Figure 33). This plant had a crown canker which extended 4 cm up the stem above the soil surface. Other plants exposed to soil sample 2 did not develop crown cankers but had substantial root decay. Plants exposed to sample 1 from the infested area did not develop crown cankers but had moderate amounts of root decay. Most of the roots that had grown out of original container soil and into the sample 1 soil were decayed. Plants exposed to soil sample 3 from healthy area showed no evidence of root decay or stem cankers. We isolated *P. cinnamomi*

from the plant with the stem canker and from decayed roots of plants exposed to infested soil sample 2, but not from plants exposed to soil samples 1 or 3.

Figure 33 *A. viscida* one month after transplanting into soil collected from Apricum Hill. Pot at left has soil from a healthy area (sample 3); center and right pots have soil from the mortality center (samples 2 and 1, respectively). One plant with two large stems in the center pot is dead.

A. myrtifolia. Rooted cuttings of *A. myrtifolia* were transplanted directly from the rooting media into field soil. Plants were allowed 11 days to adjust to transplant shock before a 12 hour flooding treatment was imposed. By one week after the flooding treatment, 3 of 8 plants in the soil samples from the mortality center were dead and all had died by 3 weeks after the flooding treatment. Many of the roots of these plants were black and decayed and no new root growth was present. The lower stems of these plants exhibited discoloration and necrosis (Figure 34). Although rooted cuttings planted in soil from the healthy area (sample Apricum Hill 3) developed some foliar chlorosis and/or reddish discoloration, most likely due to nutrient stress, none died or showed evidence of root rot. Furthermore all of these plants formed some new white roots and mycorrhizae. We isolated *P. cinnamomi* from the lower stems of all the plants growing in soil from the mortality centers, but the pathogen was not isolated from any of the plants growing in the soil from the healthy area.

Figure 34. Rooted cuttings of *A. myrtifolia* one month after transplanting into soil samples collected from Apricum Hill. The outer surface at the base of both stems has been sliced away to reveal internal tissues. Left: Dead plant with root and crown rot grown in soil collected from the mortality center. Right: healthy plant grown in soil from a healthy area. White material on roots is perlite from the original rooting medium.

DISCUSSION

At least two different diseases are affecting the health of *A. myrtifolia* and *A. viscida* in the Ione area. One is a previously identified branch canker disease caused by species of *Fusicoccum*. This disease may or may not be native to the area, and may be the disease that Adams (1934) observed on *A. myrtifolia*. The other disease, which is newly identified and documented in this report, is a root disease caused by *Phytophthora cinnamomi*. *P. cinnamomi* is not native to California and it was probably introduced into Ione formation soils in the past 20 years or so. This disease has apparently been noted since the 1980's (Roy Woodward, personal communication; Wood and Parker 1989), but it seems to have been at least partially confused with the branch canker disease up to this point. The confusion may be in part due to the fact that both diseases occur together in some areas. However, the two diseases differ in their symptoms, etiologies, and potential for impact on *A. myrtifolia* and other species. The diseases are discussed separately below.

BRANCH DIEBACK AND CANKER CAUSED BY *FUSICOCCUM* SPP.

Importance

Fusicoccum spp. on *A. myrtifolia* in the Ione area appear to function primarily as branch pathogens that may occasionally kill entire plants. Small infections that occur on branch tips probably have little impact on overall plant health and survival. Many of the infections appear to become inactive after killing individual branch tips. Other branch tip infections progress down small branches and eventually reach larger diameter stems where they may expand and girdle the stem, killing relatively large portions of plant canopy. In years or sites where conditions for disease are especially favorable, the cumulative impact of shoot tip dieback may be substantial. Cankers that do not completely girdle the stem in one year may form persistent cankers similar to those originally described by Adams (1934).

Our observations to date indicate that many stem cankers may expand rather slowly, at least between March and October. If canker expansion is typically slow, the greatest impact of cankers will be seen in plants that also are growing slowly. If the loss of branches due to cankers exceeds

the rate of new branch production and growth, plant cover will be reduced and individual plants may eventually be killed. Some plants at our lower plot at Apricum Hill and at another site off Lambert Road appear to show decline of this sort. Because *A. myrtifolia* does not exhibit crown sprouting, plants die if all of the main stems are killed. However, clonal plants derived from rooted stems may survive the death of the main stem.

***Fusicoccum* spp. description**

Fusicoccum is a form genus applied to the asexual stage (also known as the anamorph) of fungi that produce single celled fusiform asexual spores (conidia) in flask-shaped fruiting bodies (pycnidia) that are formed in host tissue. Some members of *Fusicoccum* are known to form a sexual stage (also known as the teleomorph) that is placed in the ascomycete genus *Botryosphaeria*. *Fusicoccum* spp. are distributed in temperate regions worldwide and infect a wide variety of woody dicots and some conifers.

Currently, the taxonomy of *Fusicoccum* is in flux. Recently, molecular techniques including random amplified polymorphic DNA (RAPD) markers and nuclear rDNA ITS region sequences have been used to understand the taxonomic relationships between *Fusicoccum* and allied taxa (Smith et al 2001). Results from Smith et al (2001) indicate that *Botryosphaeria dothidea*, the sexual stage of *F. aesculi,* is distinguishable from a species complex that contains *B. ribis*, *F. luteum*, and other fungi with *Fusicoccum* asexual stages. At least one California isolate identified on the basis of morphological characters as *B. dothidea* (Worrell et al 1986) has been shown to be closer to *F. luteum* based on molecular techniques (Smith and Stanosz 2001). Both *F. aesculi* and fungi in the *B. ribis-F. luteum* complex are well known in California and North America as plant pathogens that cause stem cankers and shoot blighting in various woody species. Within California, hosts of these stem canker fungi include native chaparral species (*Arctostaphlyos, Ceanothus, Garrya*, etc.), pistachio, as well as *Sequoia sempervirens* and *Sequoiadendron giganteum* trees grown outside their natural range (Smith et al 2001, Brooks and Ferrin 1994).

The *Fusicoccum* we commonly isolated from stem cankers on *A. myrtifolia*, isolate G, is most similar to *F. aesculi* in spore size, and length to width ratio (Smith et al 2001). Tim Tidwell at CDFA previously identified a *Fusicoccum* he isolated from *A. myrtifolia* as *F. aesculi* (Hartwell 2001). Our *Fusicoccum* isolate G also appears to be very similar to the *Fusicoccum* isolate that causes dieback in *Arctostaphylos* species and other chaparral plants in southern California (Brooks and Ferrin 1994). In contrast, our *Fusicoccum* isolate J, isolated from a stem canker on *A. viscida,* appears more likely to be in the *B. ribis - F. luteum* species complex based on spore size and shape. Given the current state of taxonomy in *Fusicoccum* and allied fungi, DNA sequencing of our isolates would be needed to obtain definitive identifications.

Based on the frequency of isolation from *A. myrtifolia* and the ease with which it forms spores, we believe that *Fusicoccum* isolate G may be the more important stem canker pathogen of *A. myrtifolia* in the Ione area. The relative importance of the two *Fusicoccum* spp. could vary throughout the range of the host species and could differ somewhat over time. Although we have been able to address some questions about *Fusicoccum* stem canker in *A. myrtifolia,* the length and scope of this study were not sufficient to entirely characterize this disease complex. More extensive sampling at different times of the year and over a greater geographic range is needed to determine whether additional branch canker fungi may be present on *A. myrtifolia*.

Conditions favoring disease

Fusicoccum species produce fruiting bodies in newly killed tissue in autumn. Spores are dispersed by splashing rain and new infections are most likely to be initiated during long rainy periods. It is

likely that multiple cycles of infection and sporulation can occur throughout the winter. We observed that new infections appear to start at branch tips and inflorescences, but have not directly determined the common sites of infection. Brooks and Ferrin (1994) reported that vegetative axillary buds and old terminal inflorescences were foci of *Fusicoccum* infection in *Arctostaphylos* spp. growing in southern California. We isolated *Fusicoccum* isolate G from *A. manzanita* leaves in December and have occasionally observed foliar symptoms on *A. myrtifolia* that could be due to *Fusicoccum* infection. Further studies are needed to determine whether *Fusicoccum* infects and sporulates on leaves as well as stems.

Water stress has been implicated in the literature as a factor in susceptibility of plants to infection by *Fusicoccum* and related stem canker fungi (Brooks and Ferrin 1994). Stem water potential data that we collected show that *A. myrtifolia* plants develop high levels of water stress over the summer and that levels of water stress can vary over fairly short distances. In the two plots at Apricum Hill, levels of both dieback and plant water stress were greater in the lower plot than the upper plot, suggesting a possible relationship between water stress and stem canker severity. We did not observe differences in the expansion of individual cankers that were related to plot or SWP. However, more cankers would need to be observed for a longer period in order to draw reliable conclusions about the relationship between canker expansion and water stress.

Implications for management of *A. myrtifolia*

We have observed at least low levels of branch dieback associated with *Fusicoccum* canker in all of the stands of *A. myrtifolia* that we examined. It is likely that this disease occurs at varying levels throughout the range of *A. myrtifolia*. We do not know whether the presence of other hosts such as *A. viscida* in or near the stand influences the level of disease. It is possible that sufficient disease cycling occurs within *A. myrtifolia* that the presence of alternate hosts is not necessary for disease.

In general, relatively vigorous stands that are actively growing are not likely to be substantially affected by this disease. However, stands that are growing slowly, due to advanced age, poor site quality, competition, or other factors, are more likely to be impacted by this disease complex. Treatments to initiate stand replacement and/or favor regeneration within gaps created by mortality would be the most promising means of managing severely impacted stands. However, experiments to obtain a better understanding of factors associated with regeneration in these stands are needed before such management treatments can be prescribed.

ROOT AND CROWN ROT CAUSED BY *PHYTOPHTHORA CINNAMOMI*

Importance

Root and crown rot caused by *P. cinnamomi* is a disease that kills large patches of *A. myrtifolia* and *A. viscida* in the Ione area. *A. myrtifolia* and *A. viscida* plants in all age classes may be infected and killed by *P. cinnamomi*. Because it typically kills all or a high percentage of the host plants in a given area, *Phytophthora* root and crown rot has a much more significant impact on *A. myrtifolia* than does *Fusicoccum* stem canker. *P. cinnamomi* decays both fine and large roots, and decay may extend up through the root crown. The loss of roots and/or water-conducting tissues in the root crown causes the entire plant to desiccate. Based on our pathogenicity tests, *A. myrtifolia* and *A. viscida* appear to be highly susceptible to this pathogen.

P. cinnamomi poses an extremely serious threat to the continued existence of *A. myrtifolia* populations on Ione formation soils. Because *P. cinnamomi* can persist in soils for a long (though as yet undetermined) period after death of susceptible host species, introduction of *P. cinnamomi* into the soil constitutes a long-term and possibly permanent destruction of *A. myrtifolia* habitat.

P. cinnamomi description

Phytophthora cinnamomi is a member of the Oomycota. This group of microorganisms was previously grouped with the fungi based on their overall morphology and ecological niches, although they differ from the true fungi in many significant ways. The Oomycota are now classified as a subgroup of the kingdom Chromista, which is primarily comprised of aquatic organisms, including diatoms and brown algae.

Various species of *Phytophthora* have proven to be highly destructive and important pathogens of both agricultural crops and plants in native ecosystems. *P. lateralis*, the cause of Port Orford cedar root disease, and *P. ramorum*, the cause of sudden oak death, are two introduced *Phytophthora* species that are currently impacting California ecosystems by killing important native trees. However, the impacts of these serious pathogens pale in comparison to the worldwide impacts of *P. cinnamomi*.

P. cinnamomi is a serious pathogen of both agricultural crops and native plant communities, especially forests, in temperate, subtropical, and tropical regions on all continents except Antarctica and on many islands. *P. cinnamomi* has a very wide host range including a large number of woody dicot species as well as conifers. Zentmyer (1980) estimated that over 900 plant species were susceptible to *P. cinnamomi* in his 1980 monograph, but the number of known susceptible species has continued to increase since that time. In western Australia alone, where *P. cinnamomi* has devastated several hundred thousand hectares of native forests, the host range is estimated to include over 2000 species (Wills 1993). This disease has brought a number of Australian rare plant species to the brink of extinction (Grant and Barrett 2001).

P. cinnamomi was probably introduced into California in the late 19th or early 20th century (Zentmyer 1977). In California, *P. cinnamomi* is best known as a pathogen of agricultural crops, including avocados and various orchard trees, and ornamental plants, including nursery stock and Christmas tree farms. To our knowledge, this is the first report of *P. cinnamomi* causing extensive damage in a California native plant community. We believe that several factors, including highly susceptible hosts, unique soils, and high amounts of local soil movement, probably account for the fact that *P. cinnamomi* has become an established pathogen in *A. myrtifolia* habitat.

Conditions favoring disease

P. cinnamomi survives in the soil in infected roots and as long-lived resistant spores (chlamydospores) that can survive in the soil for extended periods even in the absence of susceptible hosts. Its pathogenic activity is greatly favored by free moisture. Under moist conditions, sporangia form on infected roots. When free water is present, as in saturated soils, *P. cinnamomi* sporangia release zoospores. Zoospores are motile spores that swim through water by means of flagella. They are attracted to chemical compounds exuded by host roots, and can seek out host roots as they swim through saturated soils or flowing water. Zoospores encyst on or near host roots, sometimes in great numbers. Upon germination, zoospore cysts produce filamentous hyphae that penetrate host roots and proliferate within them, causing decay. Under wet conditions, additional sporangia can be produced on diseased roots within 48 h, so multiple infection cycles are likely to occur over the wet season.

Most new root infections occur when soils are saturated long enough for zoospores to be released and find their way to host roots, although infection does sometimes occur under moist conditions without zoospore production (Zentmyer 1980). Thus, virtually all new infections in *A. myrtifolia* and *A. viscida* are likely to occur during the wet season, which can start as early as autumn and extend as late as May.

P. cinnamomi grows best at warm temperatures between 18 and 30 C and sporangia are not typically formed at temperatures below 12 to 15 C (Zentmeyer 1980). Our isolate showed no sporangium production at 14 C in the single test we performed and low numbers of lesions developed on pear baits incubated in soil solutions at cool temperatures. Most new infections are likely to be initiated when the soil is both relatively warm and wet, as occurs during wet spring weather and, in some years, when significant rainfall occurs in the fall. Also, because warm temperatures favor growth of the pathogen within host tissues, disease may progress much faster in infected plants during these periods than in the winter. Both *A. myrtifolia* and *A. viscida* are highly drought tolerant, so visible drying of the top may lag the development of root decay. Plants affected by root rot are most likely to collapse in the late spring or early summer once evaporative demand becomes high.

Areas that remain saturated for long periods are likely to be at higher risk of disease than better drained sites. Disease is likely to develop most rapidly in low lying areas, flat or nearly flat areas with poor drainage due to clay subsoil strata, and areas along seasonal watercourses. Nonetheless, during rainy periods lasting for a day or more, even well-drained soils on slopes may remain saturated long enough for zoospore release and new infection to occur. We induced lethal root rot in *A. myrtifolia* transplanted into naturally infested field soil with regular irrigation and only a single 12 h flooding period. *P. cinnamomi* is also capable of infecting leaves and stems. Leaves and branches may become infected if they are in contact with moist infested soil or are splashed with spores from the soil surface during rainy weather, although we have not yet identified such symptoms in the field. Foliar and branch symptoms caused by *P. cinnamomi* may be difficult to distinguish visually from those caused by *Fusicoccum*.

In some soils, *P. cinnamomi* is subject to microbial antagonism that can reduce its survival and reduce disease incidence in susceptible hosts. Microbial antagonism is associated with populations of bacteria, fungi, and actinomycetes in the soil and elevated levels of soil organic matter (Weste and Vitange 1977, Downer et al 2001). Unfortunately, Ione formation soils are characterized by low pH and low organic matter content, both of which tend to suppress populations of soil bacteria. *P. cinnamomi* can function in soils as acid as pH 3 (Zentmyer 1980). These characteristics may render Ione soils at high risk for *P. cinnamomi* root rot. An analogous situation is littleleaf disease of pines in the southeastern US, which is also caused by *P. cinnamomi*. Littleleaf is most severe in pine stands that have regenerated on eroded, nutrient depleted soils of old abandoned agricultural fields.

Implications for management of *A. myrtifolia*

Two basic management strategies are critical to minimizing the impact of *P. cinnamomi* on *A. myrtifolia* populations. Preventing the spread of *P. cinnamomi* into stands that are currently free of this disease is of paramount importance for the conservation of *A. myrtifolia*. Effective containment of existing infestations to slow or stop the spread of existing mortality centers is of almost equal importance. Ideally, a third strategy would involve the rehabilitation of areas infested by *P. cinnamomi*. However, based on past experience with *P. cinnamomi* in other ecosystems, it is unlikely that *P. cinnamomi* can be eradicated from most areas where it has become established, so these areas may not be able to support sustainable stands of *A. myrtifolia* in the foreseeable future.

Restricting pathogen spread. *P. cinnamomi* and other soil-borne *Phytophthora* spp. are primarily spread to new areas through the movement of infested soil by humans. Especially during the rainy season, infested soil transported by vehicles is likely to be a major route of pathogen dispersal. Vehicles associated with activities such as mining and soil grading operations (e.g., landfills, construction sites) and off-highway recreational vehicles are of special concern due to the high

amounts of soil they may disperse compared to passenger vehicles that stay on paved roads. Stands along highways and especially those accessible by unpaved roads are at especially high risk for new introductions of *P. cinnamomi*. Washing stations have been employed in both Australia (for *P. cinnamomi*) and northern California (for *P. lateralis*) as a means of decontaminating vehicles before they leave infested areas.

Because populations of *P. cinnamomi* propagules increase during the wet season and new infections are only possible when soils are moist, the risk of pathogen spread is greatest when soils are wet. Restricting off-pavement vehicle and foot traffic in *A. myrtifolia* habitat during the wet season is therefore a key component of disease management that should be implemented immediately. *P. cinnamomi* shows low survival when exposed to long drying periods and high temperatures (Zentmyer, 1980), so the risk of casually moving viable *P. cinnamomi* propagules on feet and tires during the summer is fairly low. Nonetheless, extreme care should be taken when traveling or transporting soil or plant materials (including seed and cuttings for propagation purposes) at any time of the year. To prevent infestation of new areas, an approved testing protocol for *P. cinnamomi* should be developed for use when bulk plant material or soil will be relocated from one *A. mytrifolia* site to another.

Various materials, including 70% isopropanol, 70% ethanol, or a 10% solution of commercial bleach (=0.5% sodium hypochlorite), are effective for disinfesting contaminated footwear and equipment. If shoes or small equipment are especially muddy, it may be more convenient to place them in a plastic bag and decontaminate them in a sink offsite. Because successful decontamination procedures require special efforts and some appreciation of the nature of the pathogen, it is probably advisable to restrict access to contaminated areas to personnel that are likely to follow appropriate procedures.

In addition to spread from infested to noninfested *A. myrtifolia* stands, the pathogen can also be introduced from outside sources. *P. cinnamomi* is a common pathogen in container nurseries and can be introduced into landscaped areas from infested nursery stock. *P. cinnamomi* has also been isolated by several researchers at UC Davis from Christmas tree farms in the Apple Hill area (Placer County), so soil or plant material moved from infested Christmas tree farms within the Sierra foothills could provide another relatively local source for introduction of the pathogen. The full range of possible inoculum sources in the region needs to be determined to help formulate appropriate management actions.

Once *P. cinnamomi* has been introduced into an area, subsequent local movement of the pathogen is facilitated by water flow, so infestations tend to spread downslope and along watercourses without further human intervention. If inoculum concentrations are high enough in contaminated soil or water, animals may also play a role in spread.

Host resistance. Although selection for genetic host resistance is a common tactic used to manage *P. cinnamomi* in agricultural species, the potential application of genetic resistance in this ecosystem is limited. Due to the relictual nature of *A. myrtifolia* populations, it is possible that the genetic base represented within the species is relatively narrow. We have not yet observed clear evidence of *A. myrtifolia* plants that are resistant to *P. cinnamomi*, but we cannot rule out the possibility that plants with useful levels of genetic resistance may exist. Possible resistant plants may be identified by monitoring surviving plants in and near mortality centers, although survivors may only represent susceptible genotypes that have escaped infection.

Conservation of *A. myrtifolia* genetic resources needs to be pursued aggressively at least until studies of *A. myrtifolia* population genetics and resistance to *P. cinnamomi* can be carried out. This requires the conservation of as many viable populations and individuals as possible. Collecting and maintaining germplasm from populations threatened with extinction should also be considered.

Reducing pathogen populations. Prospects for eliminating *P. cinnamomi* from infested soils are not particularly promising. Control tactics aimed at increasing microbial antagonism through the incorporation of organic materials into the soil or mulching are not appropriate due to the nature of the plant community and its relationship to Ione formation soils. Furthermore, fire does not seem to have much potential for use in reducing soil populations of *P. cinnamomi,* due in part to the low fuel loads associated with *A. myrtifolia* stands. *P. cinnamomi* is killed in host tissues held at 50 C for 0.5 h (Zentmyer 1980). Obtaining conditions needed to obtain thermal kill of *P. cinnamomi* (uniformly heating moist soil to 50 C for at least 0.5 h to a depth of least 20 cm) would be difficult or impossible through the use of a surface fire and native fuels. Soil solarization (Pinkerton et al 2000) may be a more promising technique for killing *P. cinnamom*i thermally, although its use could only be contemplated for relatively small sites. Adverse impacts of sterilizing heat treatments on both the *A. myrtifolia* seed bank and resident mycorrhizal fungi need to be considered before such treatments are applied.

Chemical treatment may have some potential to limit the spread of established *P. cinnamomi* infection centers. Soil fumigation with a sterilant such as metam sodium may have potential to reduce *P. cinnamomi* populations in some instances (Pinkerton 2001), but will also kill other organisms in the soil, may impact water quality, and may be prohibitively expensive. Research in Australia has shown that phosphite, a relatively nontoxic salt of phosphonic acid, protects some susceptible plants from *P. cinnamomi*. Although phosphite does not eliminate *P. cinnamomi* from the soil, it may help reduce *P. cinnamomi* populations and has been used with some success in native forests in Australia. A single foliar application of phosphite at 4 g active ingredient (a.i.) per liter of water protected several highly susceptible species from *P. cinnamomi* for 2 years (Aberton et al 2001). Barrett (2001) also reported significant control of *P. cinnamomi* for 2 years from aerial phosphite application at 24 kg a.i. per ha. However, phosphite is not equally effective on all species susceptible to *P. cinnamomi* and phytotoxicity, including reduced reproductive capacity, has been observed in some species (Barrett 2001, Hardy et al 2001). Long term effects on soil properties would also need to be considered. Although phosphite is not currently registered as a fungicide in California, registration is being pursued for use against *P. ramorum*, the sudden oak death pathogen.

CONCLUSIONS

Both *Fusicoccum* stem canker and *P. cinnamomi* root rot are negatively impacting *A. myrtifolia*. Our research indicates that *P. cinnamomi* is currently a much greater threat than *Fusicoccum* and must be addressed in plans to recover and conserve *A. myrtifolia* populations. The distribution of *P. cinnamomi* within the range of *A. myrtifolia* should be mapped to learn how much of the current range is immediately threatened. Limiting the spread of *P. cinnamomi* is imperative for the continued survival of *A. myrtifolia*.

LITERATURE CITED

Aberton, M.J; Wilson, B.A.; J. Hill, J. and Cahill, D.M. 2001. Phosphite controls *Phytophthora cinnamomi* at Anglesea and Wilson's Promontory National Park, Victoria. Abstract. Second International IUFRO Meeting on Phytophthora in Forests and Natural Ecosystems. 30 September - 5 October, 2001, Perth and Albany, Western Australia. http://wwwscience.murdoch.edu.au/conf/phytophthora/prog.html

Adams, J. E. 1934. Some observations on two species of *Arctostaphylos*. Madrono 2: 147-152.

Barrett, S. 2001. Monitoring of aerial phosphite applications for the control of *Phytophthora cinnamomi* in the Albany District. Abstract. Second International IUFRO Meeting on Phytophthora in Forests and Natural Ecosystems. 30 September - 5 October 2001, Perth and Albany, Western Australia. http://wwwscience.murdoch.edu.au/conf/phytophthora/prog.html

Brooks, F. E,. Ferrin, D. M. 1994. Branch dieback of southern California chaparral vegetation caused by *Botryosphaeria dothidea*. Phytopathology 84:78-83.

Downer, A. J., J. Menge, A. and Pond, E. 2001. Association of cellulytic enzyme activities in eucalyptus mulches with biological control of *Phytophthora cinnamomi*. Phytopathology 91:847-855.

Erwin, D. C. and Ribeiro, O. K. 1996. Phytophthora diseases worldwide. American Phytopatholgical Society Press, St. Paul, MN.

Gankin, R. and Major, J. 1964. *Arctostaphylos myrtifolia*, its biology and relationship to the problem of endemism. Ecology 45:792-808.

Hardy, G. E., Barrett, S., Shearer, B. L. 2001. The future of phosphite as a fungicide to control the soilborne plant pathogen *Phytophthora cinnamomi* in natural ecosystems. Aust. Pl. Path. 30:133-139.

Hartwell, G. 2002. Survivors of the soil- rare plants of the Ione chaparral. Four Seasons, Volume II:43-61.

Pinkerton, J. N., Ivors, K. L, Miller, M. L., Moore, L. W. 2000. Effect of soil solarization and cover crops on populations of selected soilborned plant pathogens in western Oregon. Plant Disease 84:952-960.

Shackel, K. 2002. The Pressure Chamber, a.k.a. "The Bomb". UC Fruit & Nut Research and Information Center website. http://fruitsandnuts.ucdavis.edu/pressure-chamber.html.

Smith, D. R., Michailides, T. J. Stanosz, G. R. 2001. Differentiation of a *Fusicoccum* sp. causing panicle and shoot blight on California pistachio trees from Botryosphaeria dothidea. Plant Disease 85:1235-1240.

Smith, D. R. and Stanosz, G. R. 2001. Molecular and morphological differentiation of *Botryosphaeria dothidea* (anamorph *Fusicoccum aesculi*) from some other fungi with *Fusicoccum* anamorphs. Mycologia 93:505-515.

Wills, R.T. 1993. The ecological impact of *Phytophthora cinnamomi* in the Stirling Range National Park, Western Australia. Australian Journal of Ecology 18:145-159.

Wood, M.K. and Parker, V. T. 1989. Management of *Arctostaphylos myrtifolia* Parry at the Apricum Hill Reserve. Prepared for California Department of Fish and Game. Contract C-1483.6. (Issued Jan 1988, revised Jan 1989). 96 p.

Worrall, J. J., Correll, J. C., McCain, A. H. 1986. Pathogenicity and teleomorph-anamorph connection of *Botryosphaeria dothidea* on *Sequoiadendron giganteum* and *Sequoia sempervirens*. Plant Disease 70:757-759.

Zentmyer, G. A. 1977. Origin of *Phytophthora cinnamomi*: Evidence that it is not an indigenous fungus in the Americas. Phytopathology 67:1373-1377.

Zentmeyer, G. 1980. *Phytophthora cinnamomi* and the diseases it causes. St. Paul, MN: Amer. Phytopathol. Soc. Monograph 10. 96 p.