Distribution of *Phytophthora cinnamomi* within the range of Ione manzanita (*Arctostaphylos myrtifolia*)

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CONTENTS

Cover photo: Large *A. myrtifolia* mortality center due to *P. cinnamomi* at Apricum Hill, May 6, 2004.

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Abstract

We sampled soil in and near *Arctostaphylos myrtifolia* mortality centers to determine the overall geographic distribution of *Phytophthora cinnamomi* within the range of *A. myrtifolia.* Green pear fruit were placed in flooded soil samples to bait *P. cinnamomi* from the samples. *P. cinnamomi* was found in mortality centers near the northern end of the *A. myrtifolia* range near Carbondale Road. *P. cinnamomi* was also found associated with *A. myrtifolia* mortality centers between Ione and Buena Vista. No mortality centers were seen in *A. myrtifolia* stands south of Buena Vista as of June 2004.

Genetic data, based on DNA microsatellite analysis, indicated that at least three clonal lineages are represented among the *P. cinnamomi* isolates baited from mortality centers. From the geographic distribution of the genotypes represented among the *P. cinnamomi* isolates, it appears that the *P. cinnamomi* infestation is the result of multiple introductions of the pathogen into *A. myrtifolia* habitat, with subsequent local spread from points of introduction.

Baiting was also used to assess the local distribution of *P. cinnamomi* around mortality centers. *P. cinnamomi* was most reliably recovered from samples taken near the leading edge of well-defined mortality centers. It was infrequently recovered (1 of 20 samples) amid asymptomatic plants located 2.5 to 5 m beyond the edge of mortality centers. Hence, it appears that at least by early summer, the distribution of dead plants closely approximated the distribution of the pathogen in the soil.

Measurements of disease progress along permanent transects in one mortality center showed only about 0.5 m of disease spread uphill but at least 2 m of disease spread downslope after 2 years. *P. cinnamomi* was uncommonly baited from soil samples collected from the upper 1 cm of the soil profile compared with samples collected at 2-10 cm depth in May 2004, suggesting that few viable propagules of the pathogen were present on the soil surface by early summer.

Introduction

Ione manzanita, *Arctostaphylos myrtifolia,* is a rare and threatened California endemic plant that is limited to the unusual, highly acidic soils of the Ione formation in the central Sierra Nevada foothills. Significant disease problems in natural stands of *A. myrtifolia,* including death of large patches of plants, have been noted as early as 1988 (Wood and Parker 1989). In the summer of 2002, we determined that *Phytophthora cinnamomi* was the cause of a root and crown rot that has killed large patches of *A. myrtifolia* and *A. viscida* in an area of *A. myrtifolia* habitat south of Ione, California (Swiecki and Bernhardt 2003; Swiecki et al 2003). We isolated *P. cinnamomi* from symptomatic plants at two disjunct mortality centers, recovered the pathogen from soil at one mortality center using green pear baits, and completed a test of Koch's postulates to confirm pathogenicity on both hosts.

P. cinnamomi root and crown rot is a serious disease and must be addressed in plans to conserve and recover *A. myrtifolia* populations. *P. cinnamomi* has the potential to eliminate entire *A. myrtifolia* stands. Due to its persistence in the soil, it is possible that sites infested by *P. cinnamomi* will not be suitable for recolonization by *A. myrtifolia.* If this pathogen is spread to most of the Ione formation soils that constitute the current habitat for *A. myrtifolia* populations, affected habitat would be effectively lost, which would put natural populations of *A. myrtifolia* in jeopardy.

Two basic management strategies are critical to minimizing the impact of *P. cinnamomi* on *A. myrtifolia* populations. First and foremost, it is necessary to prevent the spread of *P. cinnamomi* into stands that are currently free of this disease. Secondly, and of almost equal importance, the spread of the pathogen from existing disease centers within extant stands needs to be slowed or stopped if possible. The studies reported herein were designed to provide critical information that will be needed to develop effective protocols for minimizing the spread of *P. cinnamomi* within *A. myrtifolia* habitat.

Our first priority for research was to determine the geographic distribution of *P. cinnamomi* within the range of *A. myrtifolia.* Options for controlling the spread of the pathogen depend upon how widely *P. cinnamomi* is distributed within the range of *A. myrtifolia*. To determine how widespread the pathogen has become, we used a roadside survey to note the location of possible mortality centers and confirmed the presence of the pathogen at accessible sites using soil baiting for the pathogen. In addition to surveying public rights-of-way along roads and highways, we conducted more detailed surveying by foot on public lands managed by the US Department of the Interior Bureau of Land Management (BLM), the California Department of Fish and Game (CDFG), and the County of Amador.

A second research priority was to obtain information on the local distribution of pathogen propagules around visibly diseased plants and/or mortality centers. This information will facilitate the delineation of infested areas for disease management purposes. In addition, we investigated whether the pathogen could be recovered from loose soil and debris on the soil surface within infested areas. This information is needed to assess the likelihood that the pathogen can be spread during the dry season by vehicle and/or foot traffic. Information about the local distribution of *P. cinnamomi* in soil will need to be integrated into the design of future studies to determine whether chemical control, using materials such as phosphites, may be a feasible management option.

To effectively conserve *A. myrtifolia*, it will be necessary to prevent further spread of *P. cinnamomi* from infested stands and to prevent new introductions of the pathogen into non-infested portions of the range. The relative importance of these two sources of the pathogen can be assessed by determining whether the current infestations are the result of a single introduction and subsequent spread, or multiple introductions of the pathogen. *P. cinnamomi* is found in a variety of agricultural and horticultural systems, so there are many potential avenues through which this pathogen may have been introduced into Ione manzanita habitat. Genetic analyses of isolates collected within Ione manzanita habitat has been conducted by Dr. Matteo Garbelotto's lab at UC Berkeley as part of this study. Data from these analyses and from analyses of isolates collected in other locations in California provide the basis for determining the likely routes of pathogen introduction and subsequent spread of the introduced pathogen.

Methods

Geographic distribution of *P. cinnamomi* within the current range of *A. myrtifolia*

Survey methods

Between May 6 and June 8, 2004, we conducted field surveys to assess the overall distribution of *P. cinnamomi* in accessible stands of *A. myrtifolia*. We used preliminary *A. myrtifolia* range maps developed by Tiffany Meyer (Holzman and Meyer, 2004) to locate stands of *A. myrtifolia*. *A. myrtifolia* stands included in the survey were primarily those visible from public roads. We also obtained permission to survey and sample on several publicly-owned parcels (BLM land off Carbondale Road, Amador County land west of Buena Vista Road, the CDFG Apricum Hill Preserve on Jackson Valley Road), which we accessed by foot. Areas included in the survey are shown in Figure 1. Surveyed stands were visually assessed for the presence of mortality centers that are typical of those caused by *P. cinnamomi*.

Soil sampling

Where access to mortality centers was possible, we collected soil samples from near dying and recently-killed *A. myrtifolia* and/or *A. viscida* plants. Soil samples, 105 in all, were subsequently assayed for the presence of *P. cinnamomi* by soil baiting using green pear fruit as described under "Soil Baiting" below. We took digital photographs to document conditions at each sample location. Spatial coordinates of the sample locations were recorded using a GPS receiver with WAAS differential correction (Garmin® GPS76). Soil sample locations are shown in Figure 1.

To collect soil samples, we first scraped organic debris and about 1 cm of loose soil off the soil surface. This material was not included in the subsurface soil sample, but was collected separately in some locations as noted below. After clearing the soil surface, we used the pick end of a mason's hammer to break up the soil to a depth of about 10 cm and collected soil and associated roots from the loosened soil. This portion of the soil profile typically contains the highest density of *A. myrtifolia* roots.

Soil and root pieces from the excavated hole were collected in 1 L plastic bags. At most locations, samples from two to three separate sampling sites located within about 5 m of each other were combined to produce aggregate samples of approximately 0.4 to 0.6 L. However, for soil samples collected along transects (described below), each sample was collected from a single sampling hole. Soil samples were placed in an insulated container for transport back to

the laboratory. Soil samples were held at room temperature (about 25 C) for up to 2 days before being processed for baiting (below). Sampling tools and shoes were thoroughly cleaned and disinfested with 70% isopropanol between all samples and before traveling out of known or suspected infested areas.

In addition to the samples collected in the survey, we tested five soil samples collected from an *A. myrtifolia* stand on private land that were submitted by Cameron Johnson of ECORP Consulting. These samples were collected on 4 January 2005 using the sampling protocols described above. GPS coordinates and photos of the sample sites were provided with the soil samples.

Local distribution of *P. cinnamomi* in soils near *A. myrtifolia* mortality centers

To evaluate the local distribution of *P. cinnamomi* around confirmed root disease centers, we collected soil samples along transects radiating out from discrete *A. myrtifolia* mortality centers. Along each transect, we collected soil and roots following the methods described above. Soil samples were collected 2.5 m and 5 m beyond the edge of the mortality center near asymptomatic plants; at the mortality center edge near a recently dead or dying plant; and 2.5 m from the mortality center edge toward the interior of the center, near dead plants. Transects were oriented either upslope (7 transects) or along the slope contour (3 transects) relative to the mortality center.

In addition, we collected samples 0, 10, 20, and 30 m downstream from the point of origin of a small intermittent stream. The stream's origin was at the base of one known mortality center at the Apricum Hill Preserve.

Surface vs. depth samples

To determine whether viable *P. cinnamomi* propagules were present on the soil surface during the dry season, we collected and baited paired surface and subsurface soil samples at 18 sampling sites. Surface soil samples consisted of organic debris and loose soil from the top 1 cm of the soil profile. Paired subsurface soil samples (2-10 cm depth) were collected and bagged separately as described above.

Soil baiting

We used green D'Anjou pears to bait *P. cinnamomi* from 105 soil samples collected at the sites shown in Figure 1. For soil samples that were dry at the time of collection, we initially added carbon-filtered tap water to the sample bag to adjust the soil moisture of the sample to about field capacity (about -10 to -30 KPa soil matric potential). Samples were maintained at field capacity and kept at room temperature for 1 to 2 days to promote sporangium production. This step was omitted for soil samples that were already moist, including samples collected near active watercourses and the samples that were submitted in January 2005.

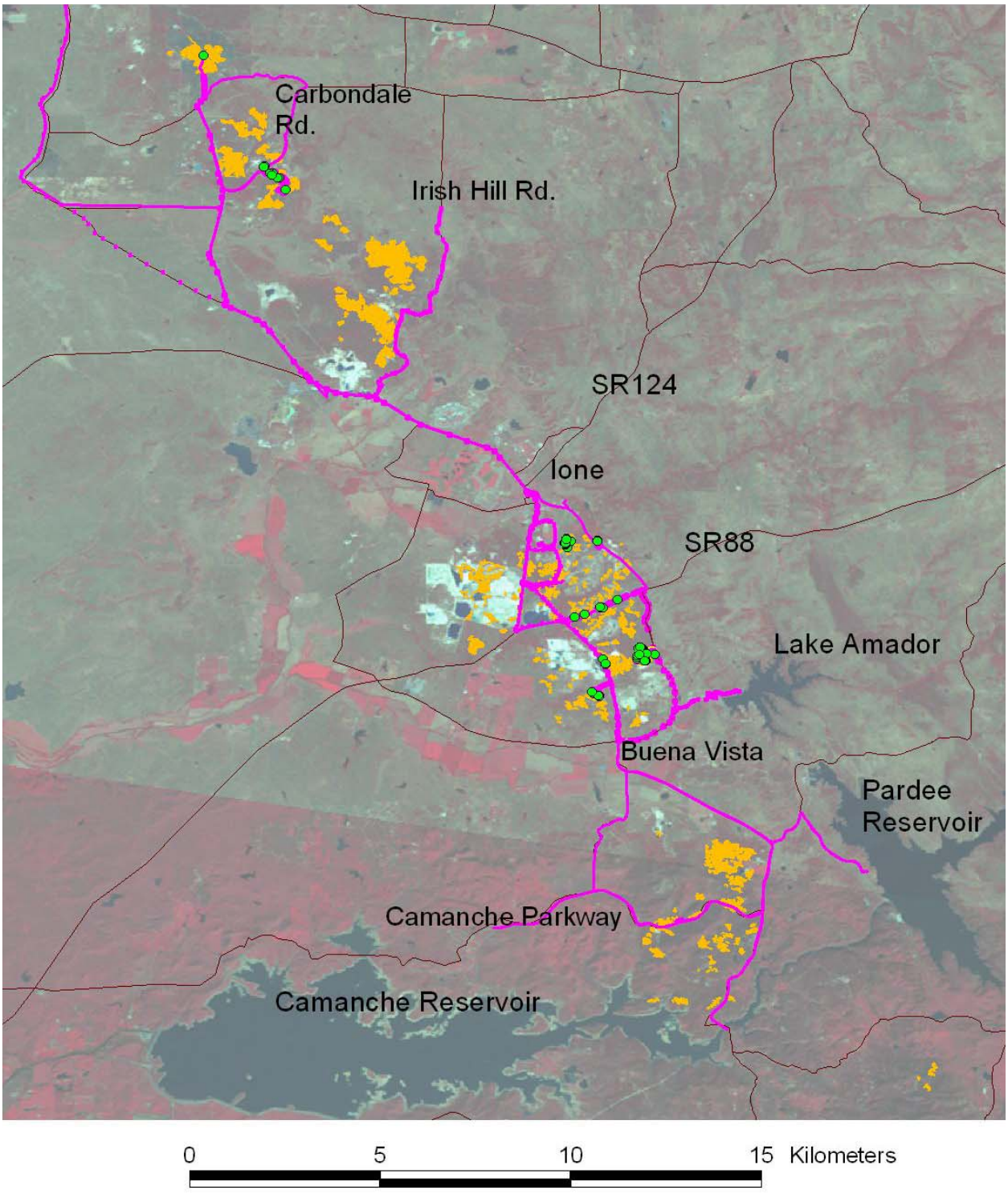

Figure 1. Survey routes (magenta lines and points) and soil sample locations (green circles) for this study shown in relation to the overall distribution of *A. myrtifolia* (orange polygons) from a GIS layer produced by Holzman and Meyer (2004). Survey routes and soil sample locations are based on GPS readings. The background image is unprojected Landsat Thematic Mapper imagery from 2000 accessed from http://seamless.usgs.gov/.

We placed one unwounded, rinsed, green pear fruit into each pre-moistened soil sample and added enough carbon-filtered tap water to submerge all but about the upper 2 - 3 cm of the pear. The water depth was about 1 to 2 cm above the soil surface in the bags. Flooded soil samples with pear baits were incubated for 1-4 days, generally until at least some baits showed symptoms. Temperatures during the incubation period fluctuated diurnally between about 19 and 31C. Pears were rinsed with water and held on racks at 20-28 C to allow for lesion development. Tissue pieces from possible *P. cinnamomi* lesions on pear baits were placed into cornmeal agar or PARP agar in petri dishes to isolate the pathogen. PARP agar, which is selective of oomycetes, consists of corn meal agar amended with 10 µg/ml pimaricin, ampicillin, rifampicin, and PCNB (Erwin and Ribeiro 1996). Petri dishes were incubated at 19-25 C in the dark to maintain the activity of the light-sensitive antibiotics in PARP. *P. cinnamomi* was identified by morphological characteristics visible through a light microscope.

DNA microsatellite analysis

Isolates from 23 different soil sample isolations were forwarded to the Garbelotto lab at UC Berkeley for DNA analyses. Genetic analysis of microsatellite markers followed the methods of Dobrowolski et al (2002).

Resurvey of disease transects at Apricum Hill Preserve

On 6 May 2004, we resurveyed three monumented transects in the first mortality center that we had documented at the CDFG Apricum Hill Preserve (Swiecki and Bernhardt 2003). The purpose of this resurvey was to assess the rate at which this disease center had expanded along the transects. The transects were established on 3 March 2002 and had been previously resurveyed 11 May 2002 and 13 October 2002. We used the point-intercept technique to rate plant cover and disease status at points spaced at 0.5 m intervals along the transects.

Results

Geographic distribution of *P. cinnamomi* within the current range of *A. myrtifolia*

Our data on the geographic distribution of the disease are limited to areas that we could observe from roadways or from vantage points on public lands. We conducted sufficient sampling to verify that *P. cinnamomi* was the likely cause of mortality in areas where patches of *A. myrtifolia* mortality were present. We isolated *P. cinnamomi* from the soil in almost all sites where we observed typical root disease mortality centers.

As of June 2004, *A. myrtifolia* mortality related to *P. cinnamomi* was observed only in areas north of Buena Vista (Figure 2). We did not observe evidence of the disease in the stands we could see in the southern part of the range between Pardee and Camanche Reservoirs. In particular, stands of *A. myrtifolia* and *A. viscida* along Camanche Parkway were generally healthy in appearance and lacked the patches of mortality seen in *P. cinnamomi*-infested areas. This agrees with assessments by BLM botanist Albert Franklin (personal communication) that noticeable areas of the disease had not been observed in that area as of that time. Nonetheless, we could only observe a small portion of the *A. myrtifolia* stands in this area, due to the hilly topography, so the presence of the disease in this area cannot be ruled out by our limited survey.

We also did not observe disease symptoms in the stand north of Lambert Road (Figure 2). Because parts of this stand were burned a few years ago, our ability to detect mortality due to *P. cinnamomi* in this area may have been impaired, but the pathogen was not detected in one soil sample collected in this area near dead plants. We also did not observe disease symptoms in stands that were visible from Irish Hill Road, but no on-site survey or sampling was conducted in the area due to a lack of permission for access.

Mortality due to *P. cinnamomi* was most common and widespread in the portion of the *A. myrtifolia* range between Ione and Buena Vista (Figure 2). We baited *P. cinnamomi* from soil with *A. myrtifolia* and *A. viscida* mortality both along roadsides and on parcels away from paved roads. Most *P. cinnamomi*-infested areas away from main roads were closely associated with unpaved roads or trails used by vehicles. In the case of the CDFG Apricum Hill preserve, which is largely inaccessible to vehicles, most infested areas are adjacent to or downslope from foot trails.

In addition, *P. cinnamomi* root disease was found along Carbondale Road in the northern portion of the range of *A. myrtifolia* (Figure 2). This area includes mortality of both *A. myrtifolia* and *A. viscida* along the roadside, localized infestations in portions of the BLM properties southeast of Carbondale Road, and a likely large, old mortality center on an east facing slope about 50-100 m west of Carbondale Road. This last area was not sampled due to lack of permission for access. Some *A. myrtifolia* mortality was also seen in the area of a development west of these disease centers along Lambert Road, but we also lacked permission to sample in this area. Because mortality in this area could be due to herbicide application or other factors, we did not assign a likely disease status to these stands.

In one area with dead and dying *A. myrtifolia* and *A. viscida* along a drainage on the eastern Carbondale BLM property (sample site 22), we recovered a *Phytophthora* sp. other than *P. cinnamomi*. This site was near trails and about 350 m south of a rural residential site. This isolate was identified as *P. cambivora* through analysis of the ITS sequence at the laboratory of Matteo Garbelotto, UC Berkeley.

Figures 3, 5, 9, and 14 show the locations of soil samples collected at a 1-10 cm depth and confirmed or likely *P. cinnamomi* disease centers. The polygons of diseased and healthy stands shown in Figures 3, 5, 9, and 14 are approximate. Patches of diseased and asymptomatic *A. myrtifolia* commonly occur adjacent to each other, even in generally infested areas, so precise mapping of the extent of mortality is very difficult. Areas mapped as *A. myrtifolia* habitat which are not denoted as either symptomatic or healthy were not assessed during the May-June 2004 survey. Photos of affected stands in these areas are shown in photos 4, 7, 8, 10-13, 15, and 16).

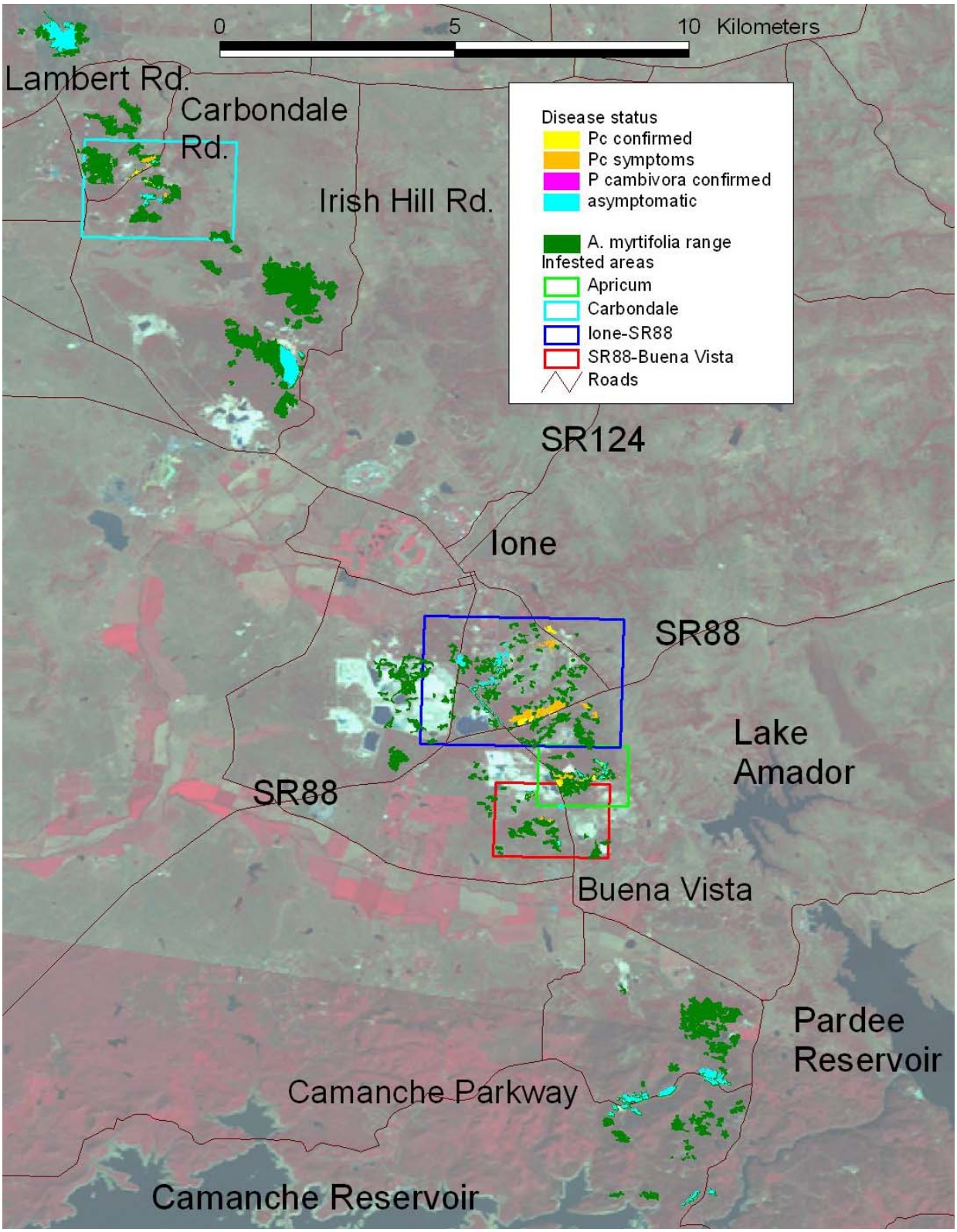

Figure 2. Disease status of *A. myrtifolia* stands throughout the surveyed area. Rectangles delimit areas with confirmed *P. cinnamomi* infestations that are shown in detail in Figures 3, 5, 9, and 14. Range of *A. myrtifolia* (dark green) is from maps produced by Holzman and Meyer (2004).

Appearance of disease centers

Some of the mortality centers had the same general appearance as those we originally described at the Apricum Hill Preserve and on the north side of SR88 (Swiecki and Bernhardt 2003; Swiecki et al 2003). These were generally long-established mortality centers, with a central area consisting of plants that had been dead for at least several years. In some cases, the plants in the oldest portions of the mortality centers had degraded entirely. At the margins of these mortality centers there were typically sharp transitions between diseased and asymptomatic plants (Figures 4, 5, 11, 12, 13). A few recently killed or dying plants were sometimes present along the outer edge of the mortality center, but many of the plants at the edge of the mortality center had no obvious disease symptoms. Small patches of asymptomatic plants were found within the outer perimeter of some mortality centers. These patches of surviving plants were commonly found on slightly raised areas within mortality centers.

Other mortality centers, which were apparently of more recent origin, did not have this well-defined structure. In these sites, dead and/or dying plants occurred singly or in small clusters throughout a localized area with intermingled asymptomatic plants (Figures 8, 10, 15). In several places, mortality was localized along drainages that are intermittently inundated during the wet season (Figures 6 [right], 8 [left], 16). The edges of some older mortality centers showed a similarly irregular distribution of diseased plants,, especially where the shrub cover was less dense and included less susceptible species such as *Adenostoma fasciculatum* and *Quercus* spp.

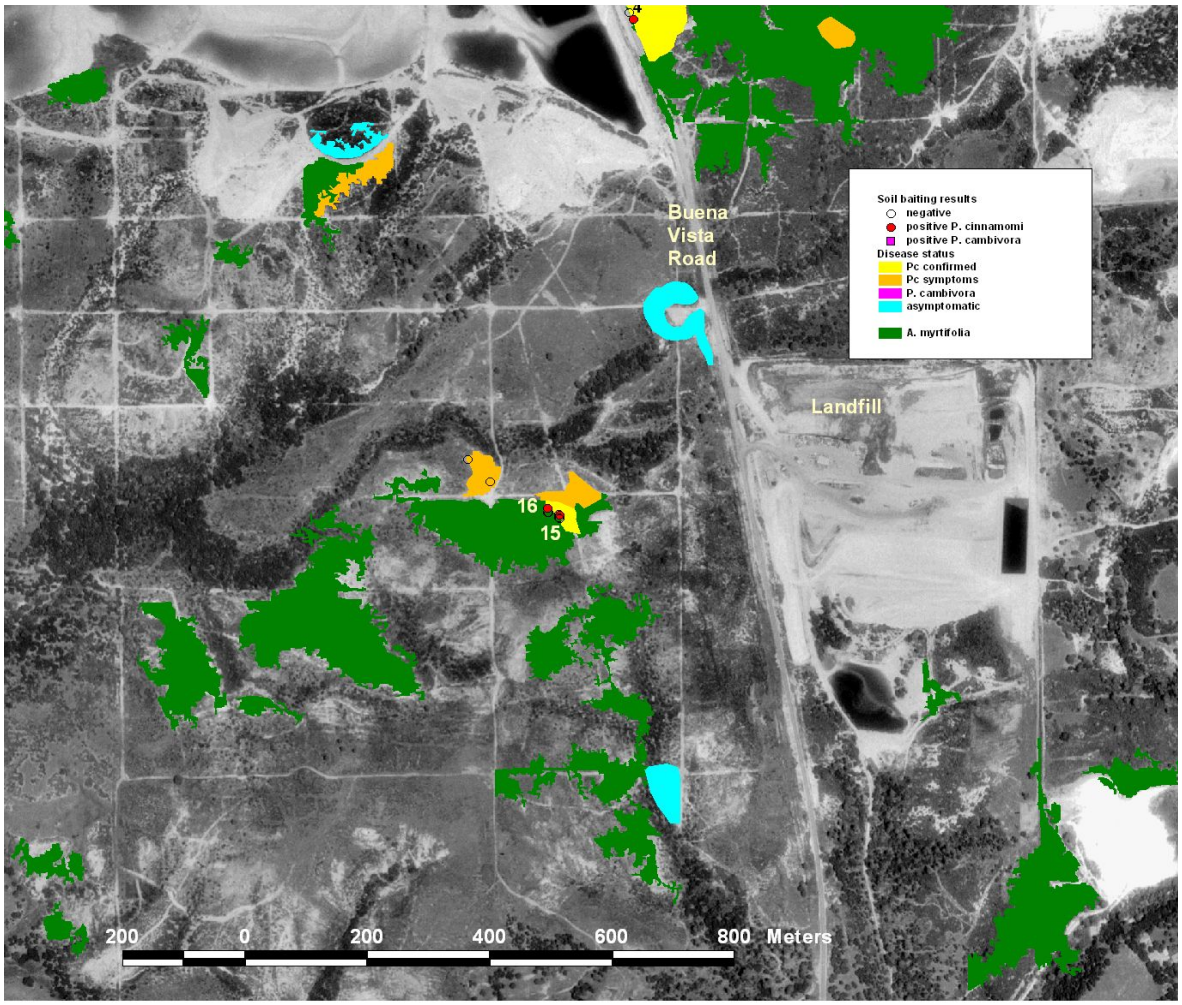

Figure 3. Disease distribution in the SR88-Buena Vista area. Mortality and sampling sites on County of Amador property west of Ione-Buena Vista Road are opposite the landfill (graded area at right). Soil sample areas 15 and 16 correspond to the number sign in Figures 4 and 5. Range of *A. myrtifolia* (dark green) is from maps produced by Holzman and Meyer (2004). The northeast corner of this figure overlaps with Figure 5.

Figure 4. Edges of old *P. cinnamomi* mortality centers in stands of *A. myrtifolia* (left) and *A. viscida* / *A. myrtifolia* (right) on County of Amador property in the SR88-Buena Vista area.

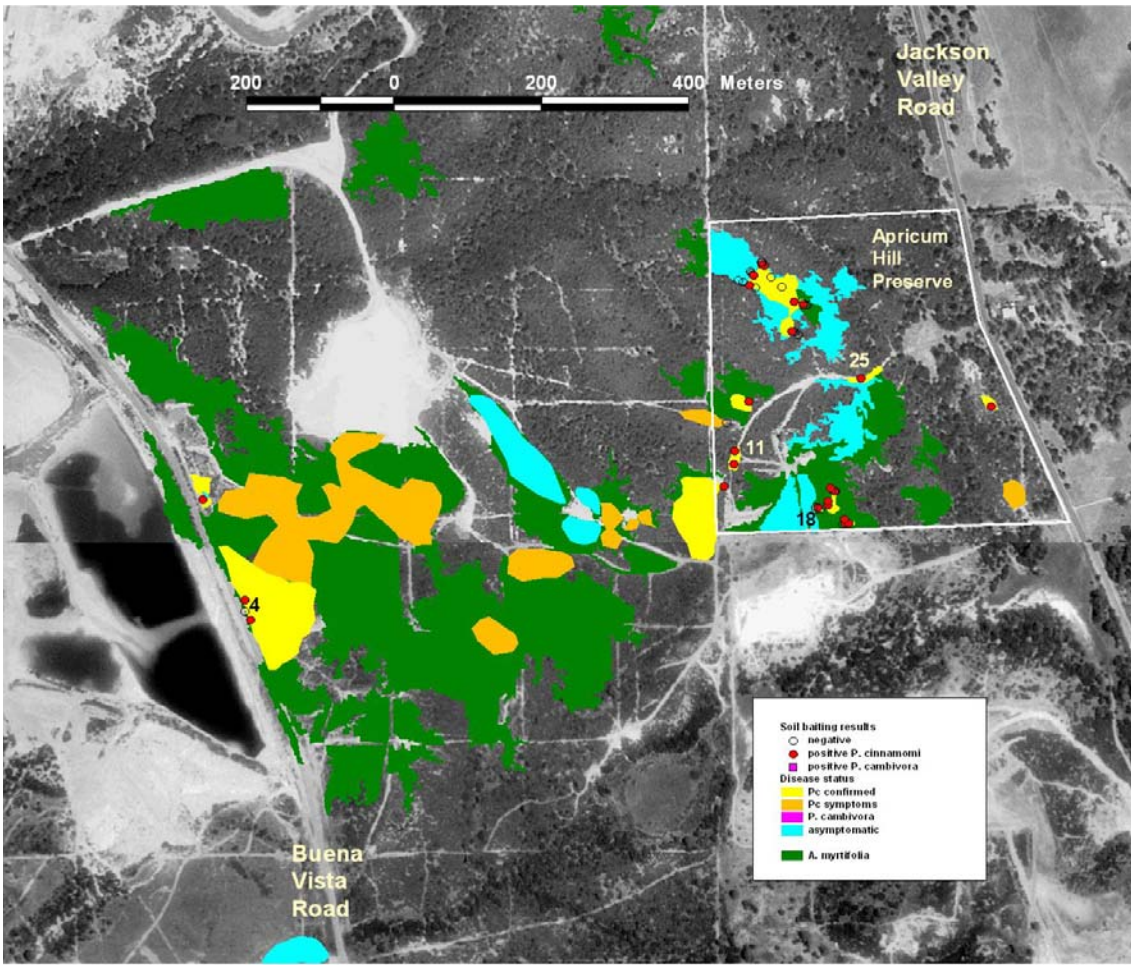

Figure 5. Disease distribution in the Apricum Hill Preserve area. Soil sample areas 4, 11, 18, and 25 correspond to the number sign in Figures 6 and 8. Range of *A. myrtifolia* (dark green) is from maps produced by Holzman and Meyer (2004). The southeast corner of this figure overlaps with Figure 3.

Figure 6. Left - Old *P. cinnamomi* mortality centers in *A. myrtifolia* stand just east of Ione-Buena Vista Road. Foreground includes recently-killed plants along the road right-of-way. Right – Relatively recent *A. myrtifolia* and *A. viscida* mortality due to *P. cinnamomi* along drainage that serves as the main path through the Apricum Hill Preserve. P

Figure 7. Likely *P. cinnamomi* mortality centers (foreground and center) in *A. myrtifolia* stand between Apricum Hill Preserve and Ione-Buena Vista Road. Ponds in background are west of Buena Vista Road.

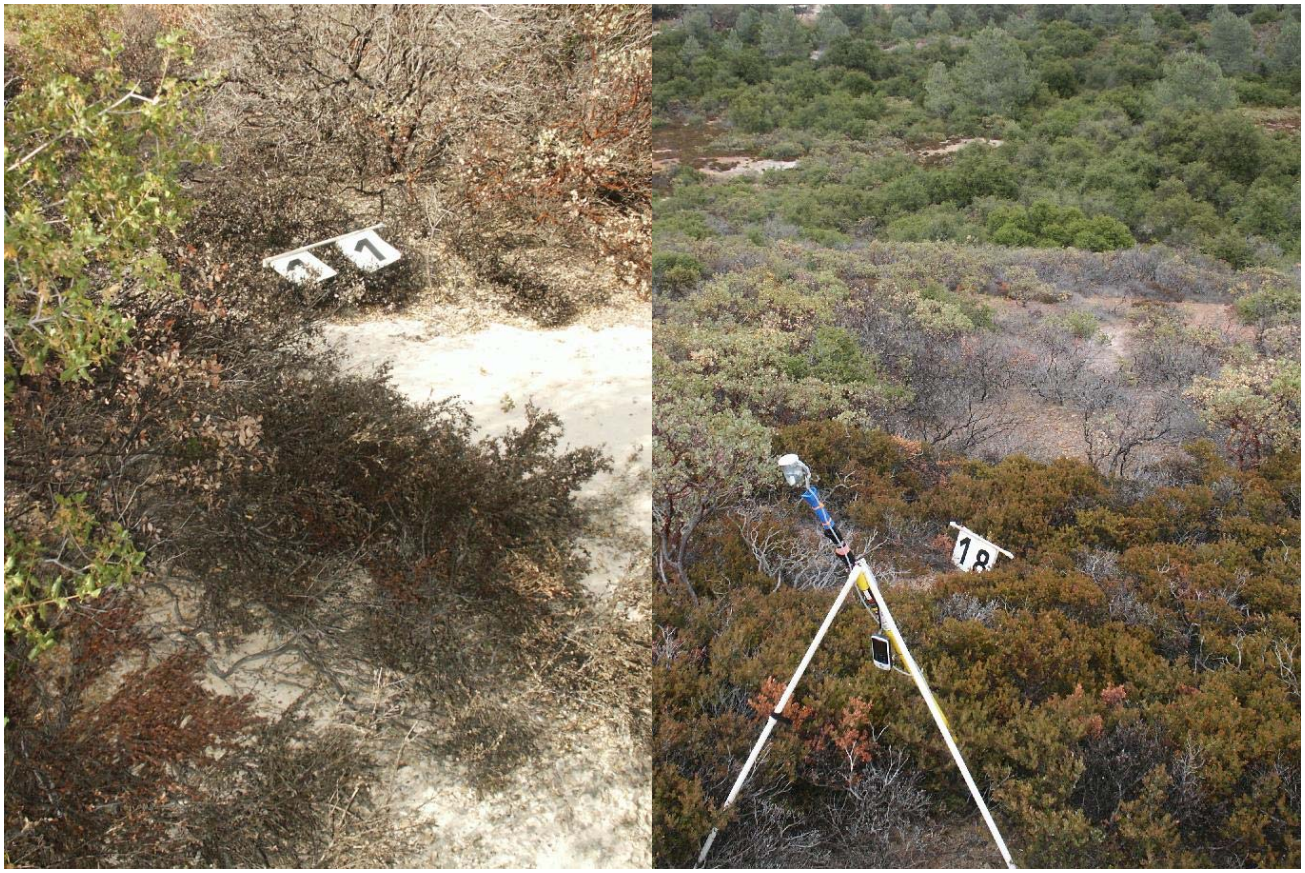

Figure 8. Small *P. cinnamomi* mortality centers in *A. myrtifolia* stands at Apricum Hill Preserve in a drainage (left) and on a slope (right).

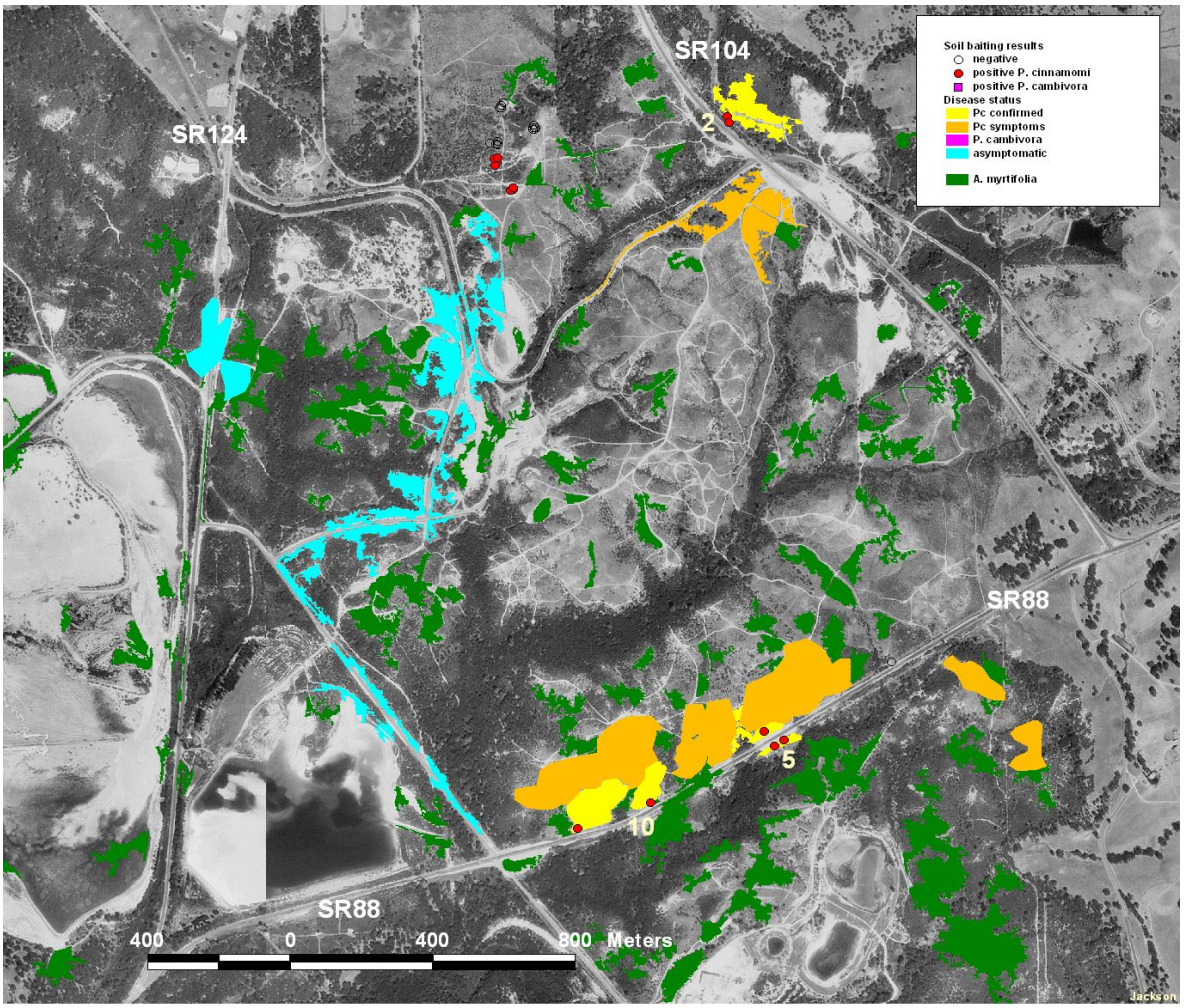

Figure 9. Disease distribution in the area between Ione and SR88. Soil sample areas 2, 5, and 10 correspond to the number sign in Figures 10, 12, and 13. Range of *A. myrtifolia* (dark green) is from maps produced by Holzman and Meyer (2004).

Figure 10. Mortality of *A. myrtifolia* due to *P. cinnamomi* on the south shoulder of SR88.

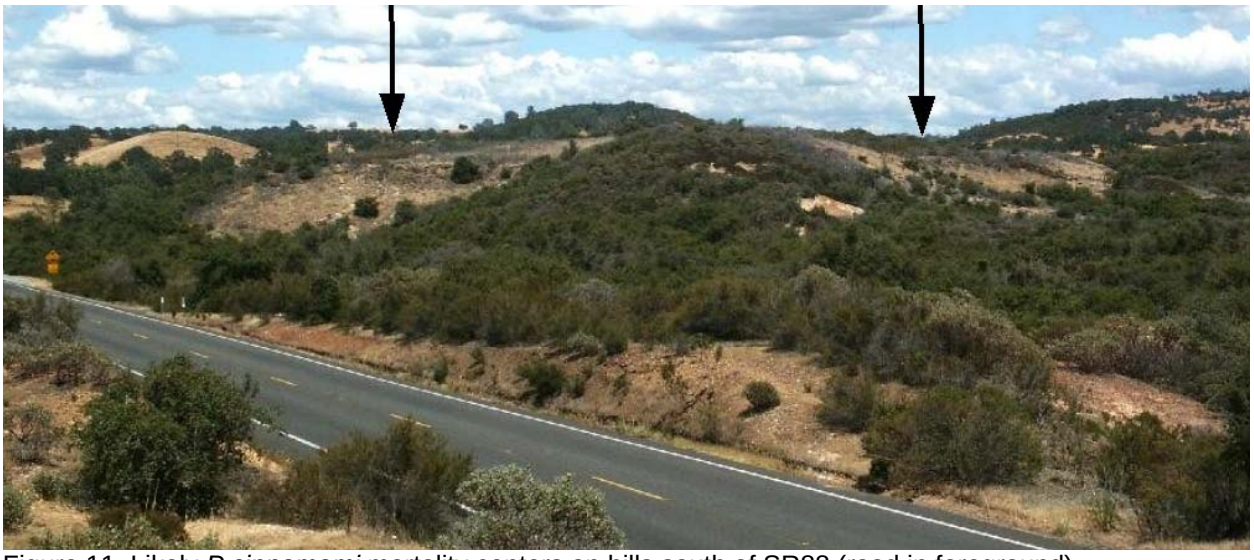
Figure 11. Likely *P.cinnamomi* mortality centers on hills south of SR88 (road in foreground).

Figure 12. *A. myrtifolia* mortality associated with *P. cinnamomi* east of SR104 (soil sample area 2 in Figure 9). .

Figure 13. *A. myrtifolia* mortality associated with *P. cinnamomi* north of SR88.

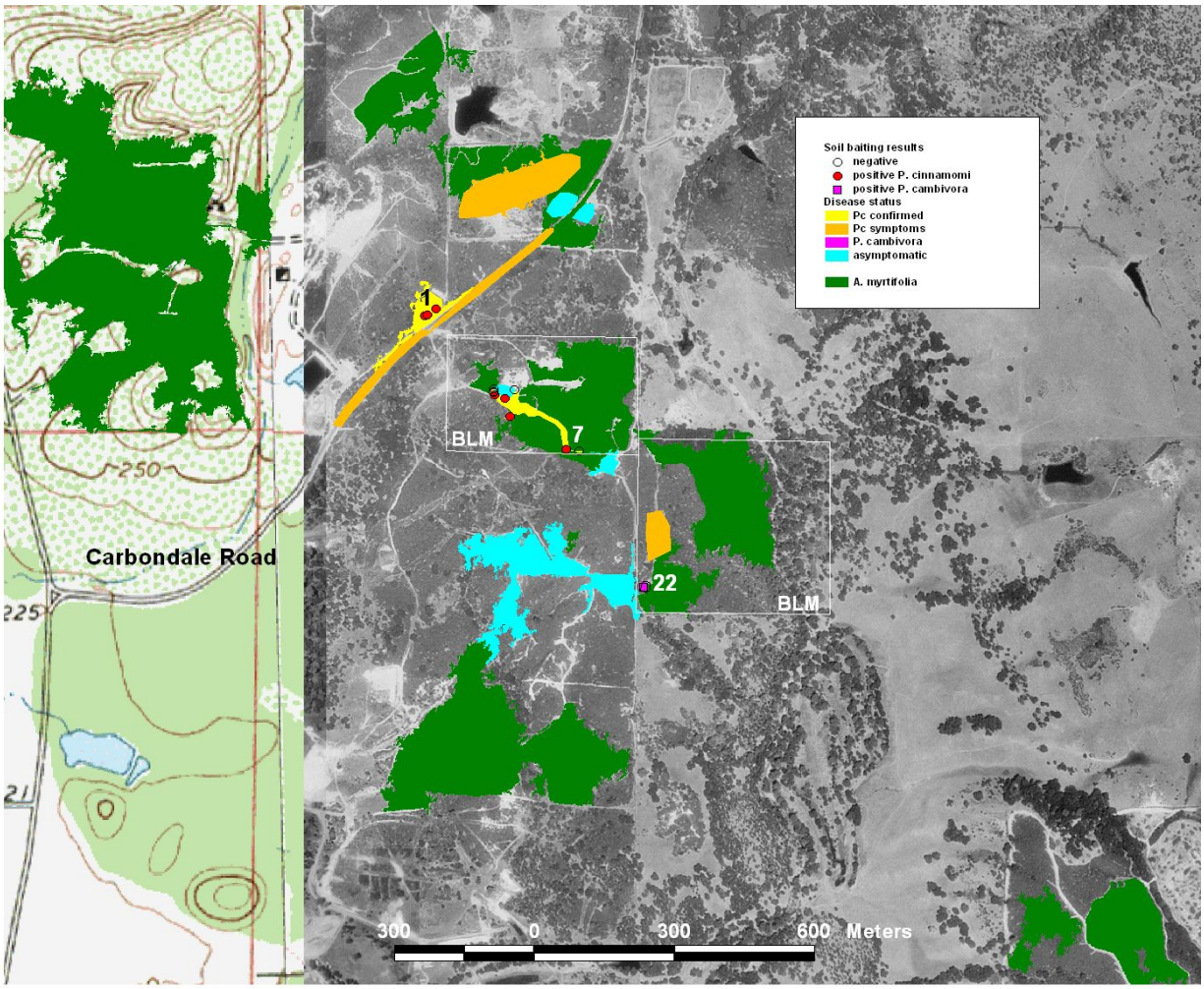

Figure 14. Disease distribution in the Carbondale area northwest of Ione. Soil sample areas 1, 7, and 22 correspond to number sign in Figures 15 and 16. *P. cambivora* was recovered from sample site 22. Range of *A. myrtifolia* (dark green) is from maps produced by Holzman and Meyer (2004).

Figure 15. Recent mortality of *A. myrtifolia* (left) and *A. viscida* (right) caused by *P. cinnamomi* on the BLM parcel near Carbondale Road.

Figure 16. Mortality of *A. myrtifolia* and *A. viscida* associated with *P. cambivora* along a drainage on the eastern portion of the BLM property.

Distribution of *P. cinnamomi* along transects near *A. myrtifolia* mortality centers

In soil samples taken along 10 transects radiating out from well-defined mortality centers, *P. cinnamomi* was most readily recovered from soil collected near dying or recently killed plants located at the advancing edge of the mortality center (Table 1). *P. cinnamomi* was recovered somewhat less reliably from the interior portion of the mortality center near plants that had been dead for an extended time. Only five of ten samples along transects that were collected 2.5 m to the inside of the mortality center edge were positive for *P. cinnamomi* (Table 1). We also baited *P. cinnamomi* from one of three additional samples collected in the interior of the large Apricum Hill mortality center (at least 5 m from the edge near long-dead plants).

In contrast, we recovered *P. cinnamomi* from only one of 20 samples taken near asymptomatic plants 2.5 or 5 m beyond the edges of mortality centers (Table 1). The single positive sample was collected along the contour of the slope 5 m from the edge of a small, relatively recent patch of mortality.

We had originally intended to orient transects upslope, along the contour (cross-slope) and downslope from *P. cinnamomi* mortality centers. However, it was difficult to establish a clear border of most infested areas in the downslope direction because the pathogen moves so readily downslope with water flow. The large, well-defined mortality centers that were used for transects either extended to the toe of the slope and/or to a change in vegetation type to a mix of species including little or no *Arctostaphylos*

To investigate inoculum movement downslope, we collected soil samples along a small seasonal watercourse that originated at the base of the largest mortality center at Apricum Hill. When samples were collected on 6 May 2004, the maximum water depth in the channel was about 10 cm and the water was not flowing. We recovered *P. cinnamomi* from soil and water collected about 10 m from the stream's origin, but not at 0, 20, or 30 m from the point of origin. Many *Quercus berberidifolia* plants growing along this watercourse showed extensive canopy thinning, chlorosis, defoliation, and dieback consistent with symptoms of *P. cinnamomi* root and crown rot. Symptomatic *Q. berberdifolia* were present adjacent to sample points 0, 20, and 30 m from the point of stream origin; *Q. berberdifolia* nearest to the 10 m sample point appeared healthy. *Q. berberidifolia* on slopes above the drainage were asymptomatic.

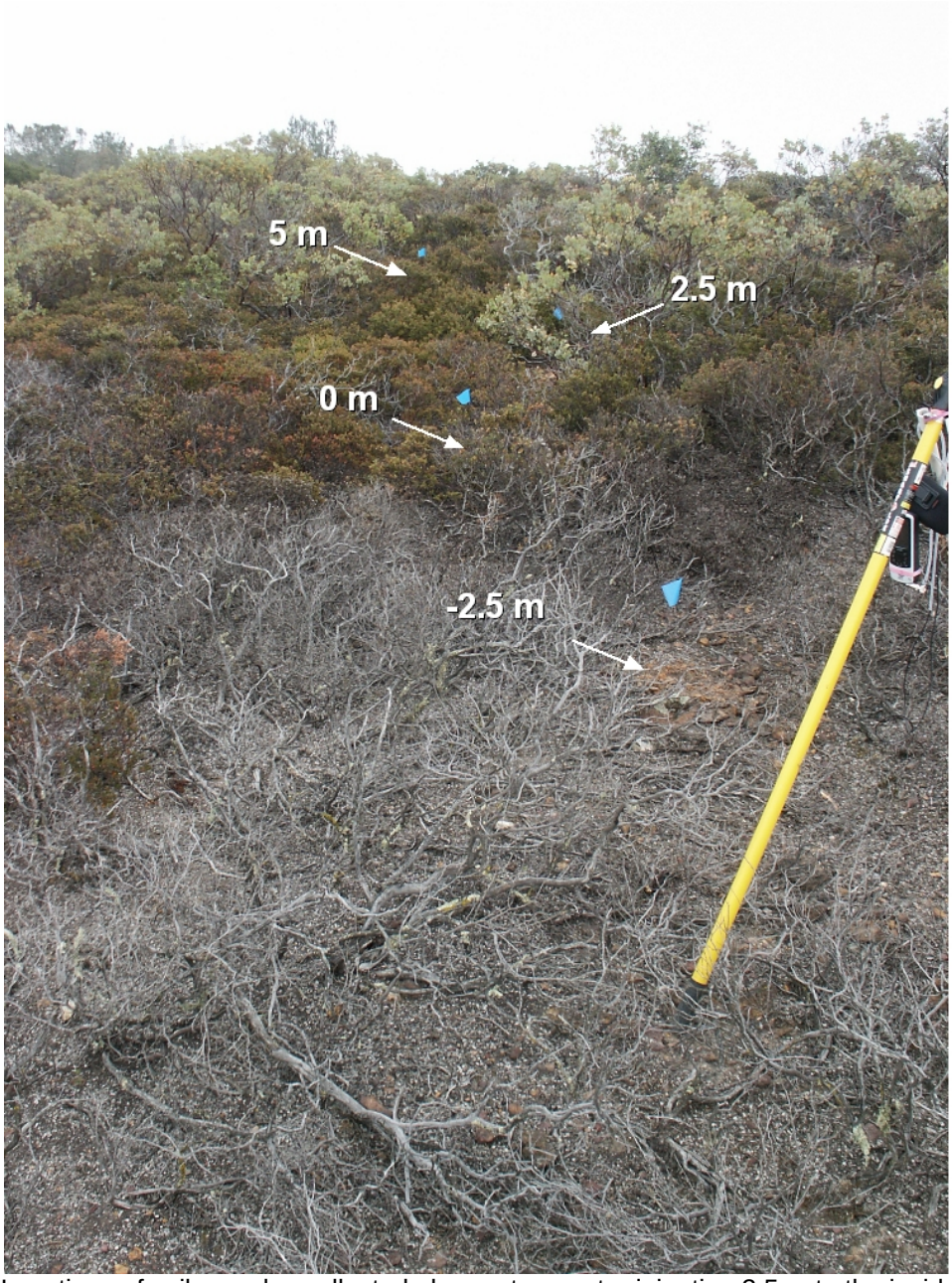

Figure 17. Locations of soil samples collected along a transect originating 2.5 m to the inside edge (–2.5 m arrow) of a *P. cinnamomi* root disease center at the Apricum Hill preserve. This transect slopes uphill away from the center of the diseased area. Blue wire stake flags mark soil sample locations.

Table 1. Detection of viable *P. cinnamomi* propagules by green pear baiting (+ = baits infected with *P. cinnamomi*, – = *P. cinnamomi* not detected) from soil samples collected along transects originating in root disease centers.

			Distance along transect[1]			
Transect Number	**Area**	**Transect orientation along slope**	**-2.5 m**	**0 m**	**2.5 m**	**5 m**
21	BLM Carbondale	no significant slope	–	–	–	–
2U	Apricum Hill	upslope	**+**	–	–	–
15	Ione-Buena Vista Rd	upslope	–	**+**	–	–
16	Ione-Buena Vista Rd	upslope	–	**+**	–	–
18	Apricum Hill	upslope	–	**+**	–	–
1U	Apricum Hill	upslope	–	**+**	–	–
26	Apricum Hill	upslope	**+**	**+**	–	–
1C	Apricum Hill	cross-slope (up)	**+**	**+**	–	–
2C	Apricum Hill	cross-slope (up)	**+**	**+**	–	–
19	Apricum Hill	cross-slope	**+**	**+**	–	**+**
Proportion of samples with positive baiting results			**5/10**	**8/10**	**0/10**	**1/10**

[1] Distance from outer edge of mortality center in the direction toward asymptomatic plants; 0 m is at the mortality center edge, -2.5 is within the mortality center. See also Figure 17.

P. cinnamomi in surface soil samples

P. cinnamomi was successfully baited from 13 of 18 (72%) subsurface (2-10 cm depth) soil samples that were paired with surface (upper 1 cm) soil samples (Table 2). In contrast, *P. cinnamomi* was baited from only one (6%) of the surface soil samples, a significantly lower percentage (likelihood ratio test $p<0.0001$). This result suggests that few viable *P. cinnamomi* were present on or near the soil surface by late May 2004. Based on data for the Camp Pardee weather station located about 13 Km southwest of Ione, the 2003-04 wet season was relatively dry. Total precipitation was 45 cm, below the 50 cm annual average. Furthermore, less than 1 cm of rain fell after March 2004, so surface soils were quite dry by late May.

Resurvey of disease transects at Apricum Hill Preserve

To monitor the rate at which a *P. cinnamomi* mortality center expands in an *A. myrtifolia* stand, we established three permanent, monumented transects in different portions of the large mortality center at the Apricum Hill Preserve in March 2002. When resurveyed in May 2004, cover ratings along these transects showed only a small increase in mortality over this time interval (Figure 18). Two transects on the upslope side of the mortality center showed very little change. At most, the edge of the mortality center advanced about 0.5 m (about the width of one small plant) into the previously asymptomatic portion of the stand along these transects. However, in the third transect, the downslope edge of the mortality center advanced at least 2 m

over the 2 year period (Figure 19). In addition to the mortality noted along the transects, we observed that some small patches of live plants within the outer perimeter of the infested area had died during this period (Figure 20). Nonetheless, some patches of live *A. myrtifolia* and *A. viscida* were still present within the mortality center. Furthermore, some live *A. myrtifolia* seedling regeneration was found in older portions of the mortality center (Figure 21).

Table 2. Number of samples with positive or negative baiting results for *P. cinnamomi* in paired samples collected from the surface 1 cm of soil and from 2-10 cm depth.

Baiting results for surface 1 cm of soil	**Baiting results for 2-10 cm depth**	**Number of samples**
Positive	Positive	1
Positive	Negative	0
Negative	Positive	12
Negative	Negative	5
Total number of paired samples		18

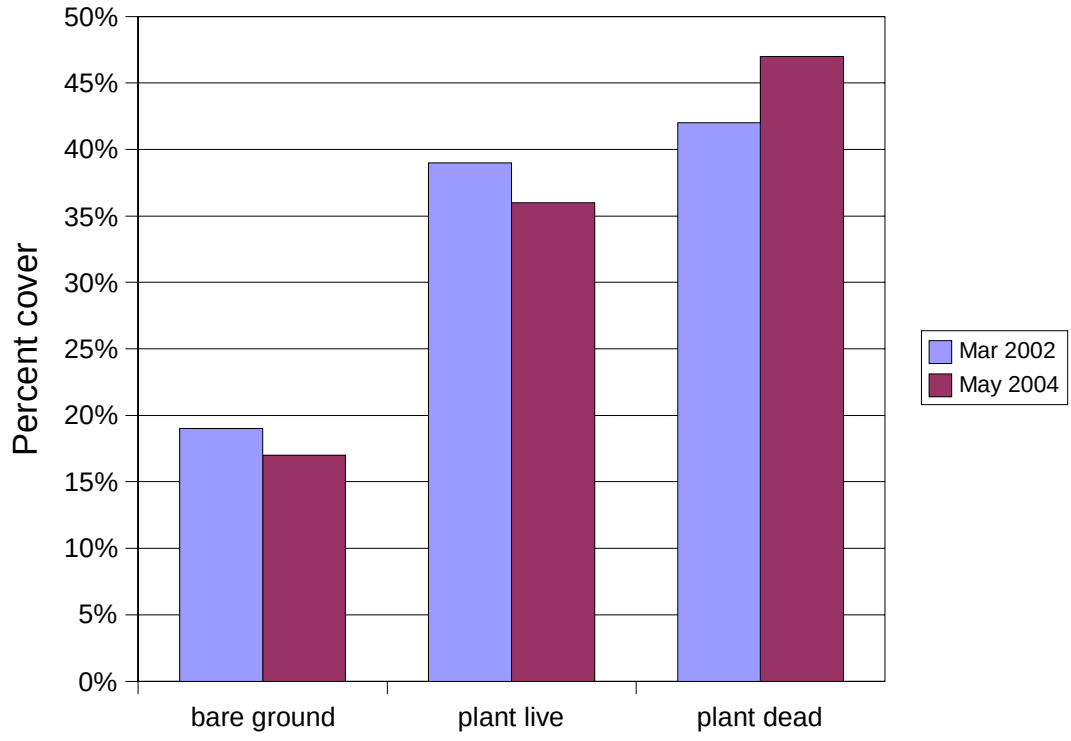

Figure 18. Cover ratings for *A. myrtifolia* and *A. viscida* (combined data) in March 2002 and May 2004 for three transects extending from the outside to the interior of the large mortality center at Apricum Hill (n=112 sample points).

Figure 19. View along permanent disease monitoring transect (measuring tape) on 6 May 2004. Dead *A. viscida* and *A myrtifolia* plants in the foreground were live when the transect was established in March 2002. The remaining patch of live plants along the transect is on a slight mound.

Figure 20. Photopoint images taken 11 May 2002 (top) and 6 May 2004 (bottom) looking toward the southeast portion of the large *P. cinnamomi* root disease center at Apricum Hill. Although the outer edge of the mortality center in this area has changed very little, some plants within the affected perimeter that were live in 2002 (top center, left of road) were dead and defoliated by 2004. Blue flags in lower photo mark soil sampling transect 1U (Table 1). One of the three permanent disease monitoring transects at this site is roughly parallel and to the left of the soil sampling transect.

Figure 21. Seedling regeneration of *A. myrtifolia* near the base of a previously-killed plant in the older portion of the large *P. cinnamomi* root disease center at Apricum Hill in May 2004.

Distribution of *P. cinnamomi* genotypes in *A. myrtifolia* mortality centers

Researchers in the Garbelotto lab used microsatellite markers to classify *P. cinnamomi* isolates from various mortality centers into genotype groups to investigate the epidemiology of pathogen introduction and spread throughout the range of *A. myrtifolia*. Although *P. cinnamomi* is known to reproduce almost exclusively asexually, various clonal lineages were identifiable. Analysis of the microsatellite markers suggested that three clonal lineages were represented in the isolates we collected from diseased *A. myrtifolia* and *A. viscida*.

One clonal lineage consisted of two variants (genotypes 1-1 and 1-2). All of the isolates from the Carbondale Road area were of genotype 1-1 (Figure 22). This genotype was also found at the Apricum Hill Preserve and in a mortality center west of Buena Vista Road south of SR88 (Figure 23). The second variant of this lineage (genotype 1-2) was also present at this latter site and in diseased plants east of Buena Vista Road (Figure 23). A second lineage was represented by a single clone (genotype 2-9) isolated at Apricum Hill Preserve along the main trail in the center of the preserve (Figure 23). A third lineage also had two variants (genotypes 3-7 and 3-8). Genotype (3-8) was only isolated at Apricum Hill Preserve, whereas genotype 3-7 was much more widespread.

All of the isolates from the large mortality center north of SR88, which is the oldest reported *A. myrtifolia* mortality center (Wood and Parker 1989), were of genotype 3-7 (Figure 23). Genotype 3-7 was also found at sample site 2 along SR104, and at most of the mortality centers at Apricum Hill, including the largest and presumably oldest mortality center (Figure 23).

Analyses completed by the Garbelotto lab showed that the DNA microsatellite marker profiles in *P. cinnamomi* genotypes 1-1 and 3-7 were also found in isolates from other parts of California, suggesting that these are relatively widespread clones of *P. cinnamomi*. In addition, three of the Ione area isolates showed the same DNA microsatellite marker profiles as *P. cinnamomi* isolates from outside California. The DNA microsatellite marker profile for genotype 1-1 was also seen in an isolate from Florida; genotype 3-7 matched an isolate from Mexico, and genotype 3-8 an isolate from China.

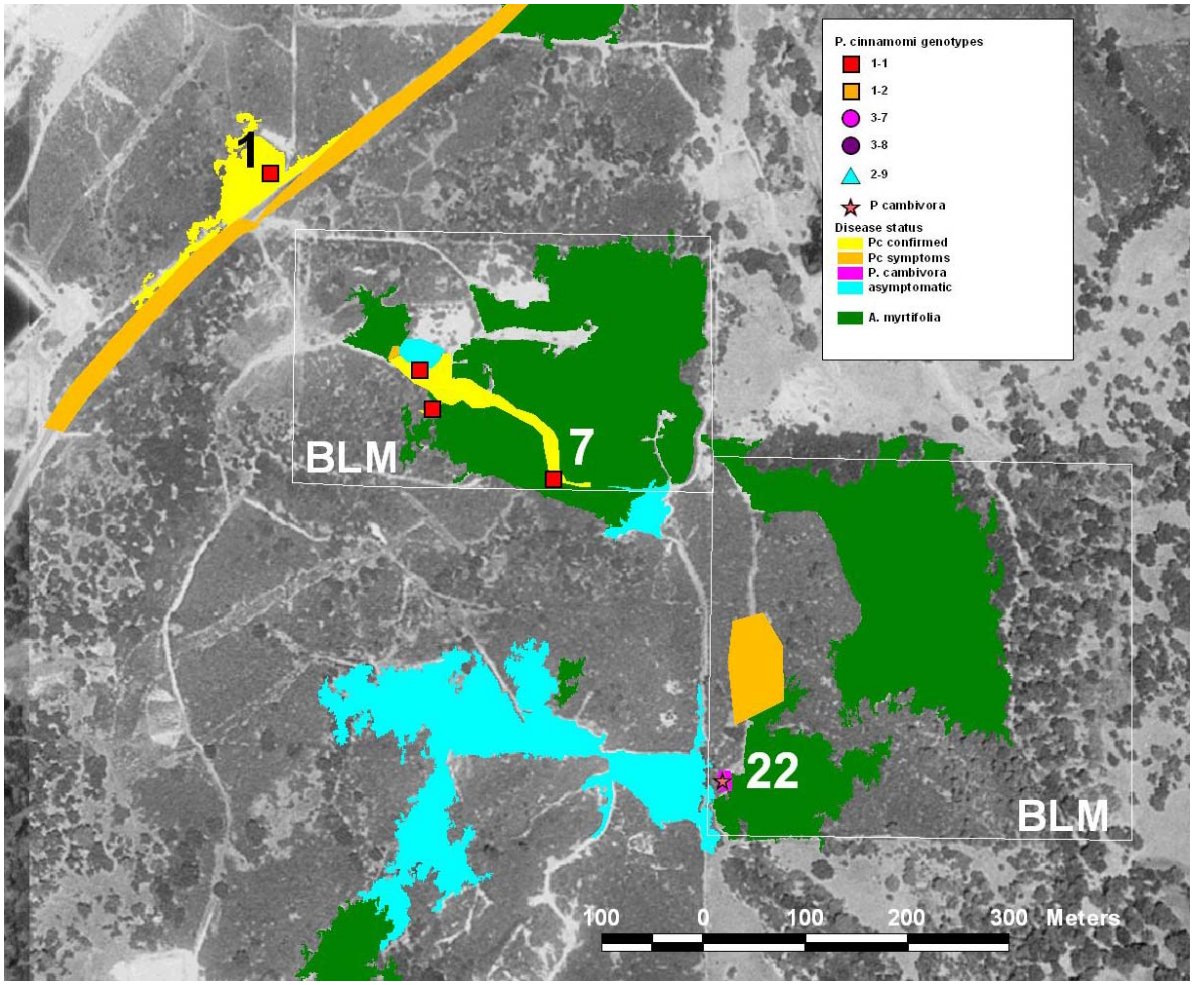

Figure 22. Geographic distribution of *P. cinnamomi* genotypes in *A. myrtifolia* mortality centers near Carbondale Road. The first number of the genotype label indicates the clonal lineage; the second is the clone number. The location where *P. cambivora* was isolated is also shown. Numbers on the map are soil sample numbers as shown in previous figures. The approximate range of *A. myrtifolia* (dark green) is from maps produced by Holzman and Meyer (2004).

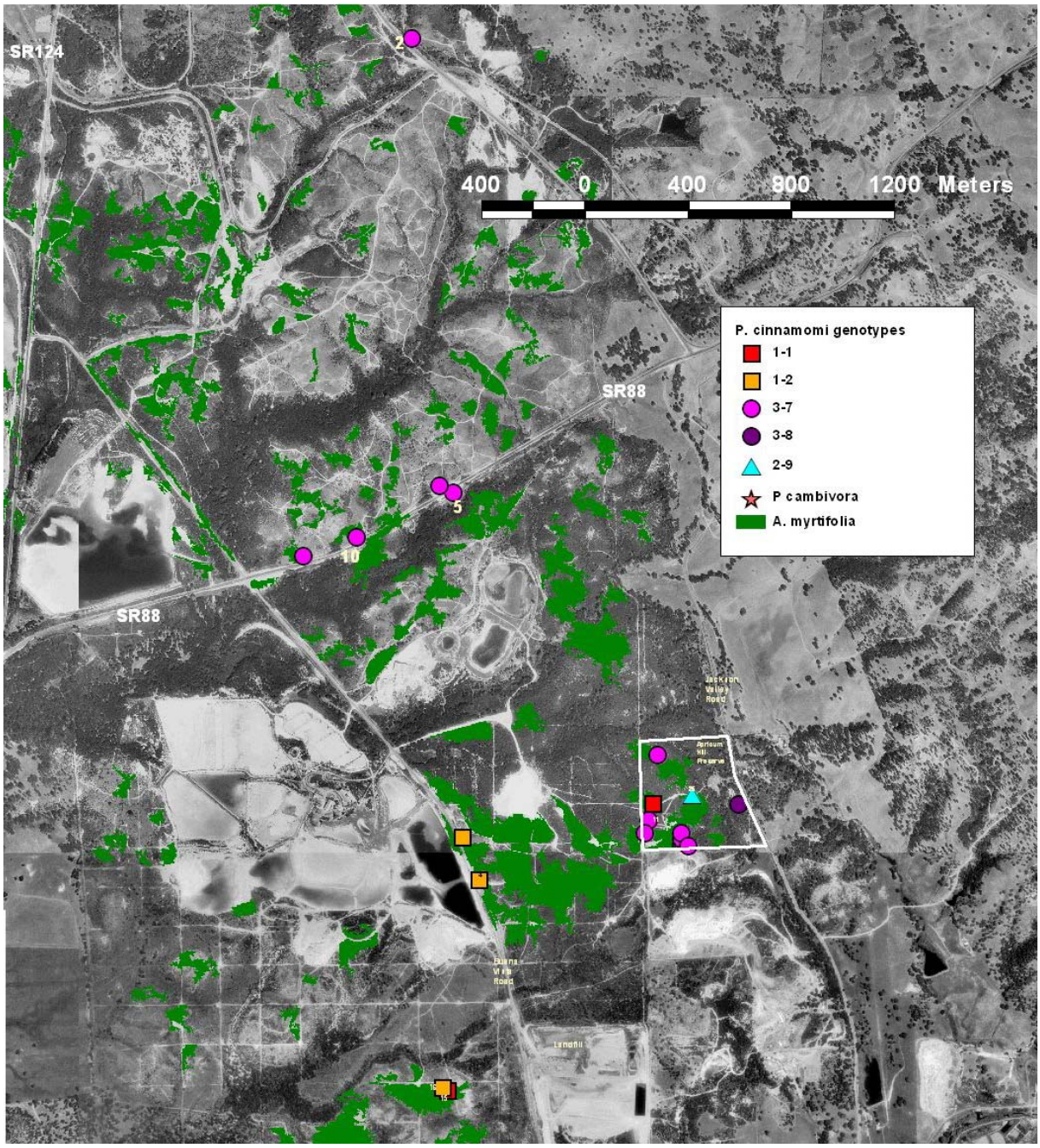

Figure 23. Geographic distribution of *P. cinnamomi* genotypes in *A. myrtifolia* mortality centers south of Ione, CA. The first number of the genotype label indicates the clonal lineage; the second is the clone number. Numbers on map are soil sample numbers as shown in previous figures. The approximate range of *A. myrtifolia* (dark green) is from maps produced by Holzman and Meyer (2004).

Discussion

Geographic distribution of *P. cinnamomi* within *A. myrtifolia* habitat

This survey documented that the *P. cinnamomi* infestation within *A. myrtifolia* habitat is relatively widespread in the area between Ione and Buena Vista, and that a separate disease locus is present in the Carbondale area. However, it is important to note that the pathogen was not found throughout the entire range of *A. myrtifolia*. At the time of the survey, the southern part of the range appeared to be free of the pathogen, although we cannot rule out the possibility that it could be in areas that we did not observe.

Because *P. cinnamomi* is not currently found across the entire range of *A. myrtifolia*, preventing pathogen spread into noninfested areas is of paramount importance. This applies not only to the portion of the range south of Buena Vista but also to areas near *P. cinnamomi*-infested soils north of Buena Vista and in the Carbondale area. New infestations can be prevented and spread from existing infested areas can be minimized by preventing the movement of soil, water, or plant material that may contain pathogen propagules. It is critically important to avoid transporting inoculum of *P. cinnamomi* into noninfested areas from either existing mortality centers or sources outside of the range of *A. myrtifolia.*

Landscape-level spread of *P. cinnamomi*

At both the Apricum Hill Preserve and the BLM parcel closest to Carbondale Road, the local spread of the pathogen appears to be primarily associated with foot traffic. Introduction of *P. cinnamomi* into previously uninfested areas along walking trails has been observed in Australia and Tasmania (Podger et al 1990, Weste and Taylor, 1971). Although access to the BLM parcel is relatively limited, the Apricum Hill preserve has been visited by a variety of researchers, agency personnel, and touring college classes over the years. This probably accounts for the greater number of mortality centers at Apricum Hill relative to the Carbondale site and the wide diversity of *P. cinnamomi* genotypes identified at Apricum Hill.

We infer that multiple introductions of the pathogen have occurred at Apricum Hill, via contaminated footwear and/or digging tools of various visitors at different times. This hypothesis is supported by the fact that many of the mortality centers at Apricum Hill are relatively small, suggesting that they may be of relatively recent origin. Also, mortality centers at Apricum Hill are scattered in areas that drain in different directions, suggesting movement by vectors such as humans rather than simply flowing water.

In addition, DNA microsatellite analyses show that *P. cinnamomi* isolates from Apricum Hill are genetically diverse (Figure 23). Four genetically distinct *P. cinnamomi* variants from three clonal lineages occur in close proximity to each other at Apricum Hill. Two of these genetic variants were not seen elsewhere among our *Arctostaphylos* isolates. Based on the clonal lineages and genetic variants present, it appears that *P. cinnamomi* has been introduced to the Apricum Hill preserve from at least three and possibly four or more separate sources, probably at different times . Two of the four genotypes identified at Apricum Hill have also been identified among isolates collected elsewhere in California.

In much of the infested area south of Ione outside of the Apricum Hill Preserve, distribution of the pathogen appears to be strongly associated with vehicular traffic. Vehicles traveling on unpaved roads during the wet season are probably responsible for most of the spread of the pathogen throughout these areas. A number of mortality centers are also found in proximity to paved roads, which suggests that these commonly serve as initial points of infestation. In Australia, the initial spread of *P. cinnamomi* through native forests was also associated with roads, especially newly graded roads (Weste and Taylor 1971, Weste and Marks 1987).

All of the isolates associated with the large mortality center along SR88 are genetically uniform, suggesting that most if not all of the mortality could be due to spread from a single pathogen introduction. It is interesting to note that *P. cinnamomi* isolates from along Buena Vista Road represent a different lineage than those along SR88 (Figure 23), suggesting that these represent two separate introductions of the pathogen to the same general geographic area. The mortality centers at the BLM parcel nearest to Carbondale Road are within a single drainage area and all isolates from this area of the same genotype (Figure 22), which implies local spread related to a single introduction. As discussed below, the *P. cambivora* infestation in the eastern BLM parcel clearly represents a separate introduction. Overall, these results suggest that spread from existing infested areas and new introductions from beyond the infested area both play significant roles in the infestation of *A. myrtifolia* habitat with *P. cinnamomi*.

Sources of *P. cinnamomi* genotypes found in the Ione area

The *P. cinnamomi* isolates recovered from *A. myrtifolia* habitat show a relatively high level of genetic diversity. Genetic data indicate that at least three distinct introductions of the pathogen have occurred. Furthermore, the fact that multiple genotypes have been able to survive and cause disease within *A. myrtifolia* habitat suggests that this environment is extremely favorable for the pathogen.

Because *P. cinnamomi* is commonly found in both agricultural and horticultural systems, the pathogen could have been introduced from any of a number of sources. Genetic variants from two of the clonal lineages identified in *A. myrtifolia* stands (1-1 and 3-7) were also found in Christmas tree farms in the general area. Given that Christmas tree farms experience high volumes of traffic during December, when soils are usually wet, it is easy to imagine that viable *P. cinnamomi* propagules could be transported to roadsides within *A. myrtifolia* habitat via infested soil or plant material. The third clonal lineage found in *A. myrtifolia* habitat is associated with ornamental plants. Soil and/or water from infested ornamental plants planted in local landscapes and diseased plant material dumped along roadsides (which we have observed in the area) represent potential avenues through which *P. cinnamomi* associated with container nursery plants may have been introduced into *A. myrtifolia* habitat.

The Garbelloto lab also identified genotype 3-7 as the same genotype responsible for the extensive oak mortality which occurred in a natural forest in Mexico (Tainter et al 2000). This is the first report of this genotype occurring outside of Mexico. This genotype may represent a particularly aggressive strain of the pathogen, and could therefore represent a threat to other natural ecosystems in Cailfornia and elsewhere. Additional pathogencity testing is needed to determine whether all clonal lineages are equally aggressive in causing disease to *A. myrtifolia* and associated native plant species.

Genetic variants 1-2 and 2-9 did not match any other isolates from the worldwide *P. cinnamom*i collection which have been analyzed to date. These variants may represent mitotic recombination and/or mutation which occurred after introduction of the fungus to the area, or they may represent separate introductions from sources not represented among the isolates used in the DNA microsatellite analysis. Analysis of additional isolates from outside the Ione area may reveal if these genotypes are unique to the Ione area.

Due to the possibility of mitotic recombination between different genotypes of the pathogen, care should be taken to avoid the movement of the pathogen between already infested areas. Recombinants may have greater levels of virulence, wider host range, or better survival characteristics, which could further complicate disease management and may increase the threat to other California plant communities. This issue is most critical at the Apricum Hill Preserve, which already has a wide variety of *P. cinnamomi* variants represented, some of which may be recombinant in origin. Intensive efforts should be made to minimize the spread of the pathogen within and from Apricum Hill, including the possible use of phosphonate (a selective fungicidal material).

Local distribution and spread of *P. cinnamomi*

Results from sampling along transects radiating out from established mortality centers indicate that levels of *P. cinnamomi* inoculum in the soil are typically undetectable by baiting even a few meters beyond the last symptomatic plant in directions upslope or along the slope contour relative to the mortality center. Similarly, in Tasmanian plant communities affected by *P. cinnamomi* that had sharp boundaries between diseased and healthy vegetation, Podger et al (1990) found that samples from diseased vegetation invariably tested positive for *P. cinnamomi*, and healthy uphill vegetation almost always tested negative. The few exceptions occurred where samples were collected less than 2 m from the boundary between diseased and healthy foliage.

The limited data we have from disease progress transects and photopoints near the large Apricum Hill mortality center show that *A. myrtifolia* and *A. viscida* plants attacked by *P. cinnamomi* can transition from asymptomatic to dead and defoliated within two years. This is not surprising, given that both *A. myrtifolia* and *A. viscida* are highly susceptible to the pathogen (Swiecki and Bernhardt 2003; Swiecki et al 2003). The fact that *P. cinnamomi* was exclusively isolated from near symptomatic plants at the edge of well-defined mortality centers provides further evidence that plant mortality typically occurs fairly quickly, within a year or so, once *P. cinnamomi* inoculum levels in the upper 10 cm of the soil are high enough to be detected by baiting.

Within the disease progress transects in the large Apricum Hill mortality center, we found very little expansion of mortality in the upslope and cross slope directions over a two year period. Expansion upslope and cross slope was about 0.25 m per year. We have also noted that at most well-defined mortality centers, only a few dying or recently-killed (i.e., with dead leaves still attached) plants are typically found along the leading edge of the mortality center. These dead plants are commonly scattered singly along the edge of the mortality center. Given that most *A. myrtifolia* plants are less than 1 m in diameter in these stands, observed mortality patterns are consistent with rates of disease spread of less than 1 m per year in upslope and cross-slope directions.

Given the low rates of disease spread and limited distribution of *P. cinnamomi* propagules at the upslope and cross-slope margins of mortality centers, it is likely that disease spread in these directions occurs primarily by plant to plant spread via infected roots. Under this scenario, roots from healthy plants become infected through direct contact with other infected roots, by hyphae growing from infected roots, by chamydospores in the soil, or by zoospores traveling short distances within the soil from infected roots to nearby healthy roots. The pathogen then moves further into the uninfested zone primarily by growing through infected roots toward the root crown. This method of disease spread has been proposed to explain low rates of *P. cinnamomi* advance (0.77 to 1.3 m/year) in Australian plant communities composed of highly susceptible plant species growing on well-drained soils (Hill et al 1994).

Given these results, we suggest that around well-defined, long-established mortality centers a buffer of 5 m should generally be adequate in the upslope and cross-slope directions for purposes of establishing local quarantine areas to avoid pathogen spread or for possible fungicide treatments areas. We did recover *P. cinnamomi* at a distance of 5 m from a mortality center edge in one transect, but this was a small disease center that had developed relatively recently. In such areas, the extent of mortality may not reflect the distribution of the pathogen as precisely as in older disease centers because obvious disease may not yet have developed around all points where the pathogen was initially dispersed. Larger buffers, perhaps 10 m from the apparent edge of the mortality center, may therefore be appropriate for quarantine and/or treatment buffers around more recent, less well defined mortality centers.

We were not able to clearly define the downslope edge of any of the mortality centers we investigated. The mortality centers either extended to the toe of the slope and/or to a change in vegetation type downslope and therefore did not provide a sharp transition between diseased and asymptomatic *Arctostaphylos*. Furthermore, in our disease progress transect at Apricum Hill, we observed higher rates of spread in the downslope direction (at least 2 m in two years) than in the upslope and cross slope directions (about 0.5 m in two years). Zoospores from infected roots that are transported downslope with flowing water presumably account for the more rapid spread of the disease downslope from infested areas.

These results are consistent with various studies of disease spread in forests or orchards infested with *P. cinnamomi* and other soil-borne *Phytophthora* spp. Inoculum of soilborne *Phytophthora* spp. moves readily downslope from infested areas with water flow, but the rate of spread upslope or along a slope contour is generally slow in well-drained soil unless soil is transported from an infested area through human activities (Hill et al 1994, Weste and Law 1973, Weste and Taylor, 1971, Zentmyer 1980). Australian researchers have shown rates of downslope spread as high as 400 m per year (Weste and Marks 1987). Slope, vegetation, the timing and amount of precipitation, and the presence of watercourses are all likely to influence the rate of downhill spread of *P. cinnamomi* in *A. myrtifolia* stands.

Under stagnant stream conditions, we recovered *P. cinnamomi* 10 m downstream from a mortality center. Viable zoospores of *P. cinnamomi* and other *Phytophthora* spp. can typically move downstream in flowing water (Oudemans, 1999). Long range dispersal of *P. cinnamomi* may therefore occur in seasonal watercourses during the wet season. Furthermore, *Q. berberidifolia* growing at least 40 m from the mortality center along this stream showed symptoms consistent with *P. cinnamomi* root rot. In contrast, *Q. berberidifolia* growing on *P.*

cinnamomi infested slopes did not show obvious root disease symptoms. We also observed mortality of trees growing in a riparian area that drains the heavily-diseased area south of SR88. Although we have not investigated these trees, it is possible that the observed mortality is also associated with *P. cinnamomi*. Further studies are needed to assess the timing and extent of pathogen dispersal in streams and the role of other host species as potential sites of inoculum production.

P. cinnamomi was less frequently baited from soil samples collected near old dead plants in the interior portions of mortality centers than it was from samples taken at the edge of mortality centers (Table 1). The presence of healthy *A. myrtifolia* regeneration within older portions of mortality centers (Figure 21), which serve as *in situ* baits, provides further evidence that viable *P. cinnamomi* propagules are not present at uniformly high densities near plants that have been dead for a number of years. However, we have previously observed mortality due to *P. cinnamomi* in *A. myrtifolia* regeneration that had become established in an older portion of a mortality center (Swiecki and Bernhardt 2003). Although reductions in pathogen populations may occur over time in old mortality centers, it is not clear how long it would take for *P. cinnamomi* populations to drop to levels that would allow for successful reestablishment of *A. myrtifolia* stands.

Australian researchers have shown that *P. cinnamomi* inoculum is not distributed uniformly in old disease centers (Pryce et al 2002, Dawson et al 1985). Furthermore, populations of *P. cinnamomi* declined over a 15 year period in infested quadrats within affected Australian forests, and regeneration of some susceptible species became established over the same period (Weste et al 2002).

P. cinnamomi was detected in only one of the surface soil samples, which were collected in May and early June 2004. Hence, at least by the beginning of the dry season, the number of viable *P. cinnamomi* propagules present on or near the soil surface was apparently low. In years with greater amounts of late season precipitation (e.g., spring 2005), *P. cinnamomi* inoculum density at the soil surface might be higher in late spring, but additional sampling would be required to test this hypothesis.

Nonetheless, the surface soil sample data indicates that *P. cinnamomi* inoculum density is low in dry surface soil. Because only very small amounts of dry soil are transported on shoes and vehicle tires, we believe that walking over contaminated soil during the summer is unlikely to be a major mode of pathogen dispersal. However, creeks and other areas that remain wet in the summer may serve as a source of readily-dispersed inoculum in the dry season. In addition, we readily isolated *P. cinnamomi* from soil samples collected to a depth of only 10 cm during the dry season (Table 2). Hence, any activity that moves surface soil, e.g., grading, mining, or construction activities, could be an important mechanism of pathogen dispersal during the dry season. To prevent further spread of the pathogen, it is necessary to decontaminate soil-moving equipment and avoid moving soil from infested areas at all times of the year.

During the wet season, even light foot and vehicle traffic can move substantial volumes of soil long distances, especially from areas where sticky clay soils are present at the soil surface. It is also possible that higher inoculum concentrations may be present near the soil surface during the wet season, although this assumption has not been verified for Ione formation soils. Thus, the

risk of disease spread by human activities is much greater in the wet season than in the dry season. Spread of the pathogen from infested areas may be slowed by restricting entry into diseased areas to the dry season where possible, and disinfesting footwear and vehicle tires when leaving infested areas. Although the risk of moving the pathogen by walking or driving on infested soil may be lower in the dry season than in the wet season, driving or walking in infested areas should be avoided if possible even in the summer and shoes and tires should be disinfested upon leaving infested areas at all times of the year.

Mapping *P. cinnamomi* in *A. myrtifolia* habitat

Polygons showing the presence or absence of *P. cinnamomi* root rot in the GIS layer (Figures 2, 3, 5, 9, and 14) were based on GPS coordinates within surveyed areas. For stands that could not be visited but were visible from various vantage points, approximate polygons were sketch-mapped on topographic maps with the aid of compass bearings and photographs. For the most part, we generated new polygons to illustrate disease centers rather than using the Holzman and Meyer (2004) *A. myrtifolia* distribution GIS layer.

Holzman and Meyer (2004) reported that 30% of their *A. myrtifolia* polygons, which were developed from multispectral aerial imagery, were misclassified with respect to the presence of *A. myrtifolia*. Due to the presence of misclassified polygons, it was necessary for us to create new polygons for some areas. Furthermore, the Holzman and Meyer *A. myrtifolia* polygons do not include large areas where the previous *A. myrtifolia* stand has been killed by *P. cinnamomi,* and therefore underestimate the extent of the infestation. For example, much of the extensive mortality center just north of SR88 (Figures 9-13) was not mapped by Holzman and Meyer (2004) as *A. myrtifolia* habitat even though remnant dead stems show that *A. myrtifolia* was distributed over much of this area before the pathogen was introduced. For disease management purposes, it is important to document the extent of the *P. cinnamomi*-infested soils within the region even if some of the soils do not currently support populations of *A. myrtifolia*. For this reason, our disease polygons also include some areas of manzanita mortality near *A. myrtifolia* stands that include *A. viscida* but little or no *A. myrtifolia*.

P. cambivora as a second root pathogen of *Arctostaphylos* in the Ione area

An unexpected outcome of this survey effort was the identification of a second soil-borne *Phytophthora*, *P. cambivora*, associated with root disease of *A. myrtifolia* and *A. viscida* in the Carbondale area. Although this pathogen was isolated in only one area near a seasonal creek, we observed an area with symptomatic *A. viscida* just upslope from the location where this pathogen was isolated (Figure 23). Further sampling in this area and adjacent areas will be needed to determine how large an area is affected by this pathogen.

P. cambivora is closely related to *P. cinnamomi*, and is also an aggressive root pathogen with a wide host range. *P. cambivora* is naturalized in parts of Europe, where it causes ink disease of chestnuts (*Castanea*), and has been shown to be pathogenic to oaks and beech (*Fagus sylvatica*) in forests. It has been found affecting nursery stock of various species in both Europe and the US. In some Oregon forests, *P. cambivora* causes a lethal canker disease of chinkapin (*Castanopsis*). It also causes a collar rot of almonds, cherries, and apple, and has been reported on Noble fir in Christmas tree farms in Washington.

Given that the area where *P. cambivora* was detected is well away from paved roads, the most likely sources of introduction are via off-road traffic along unpaved roads in the area or via inoculum that was carried down the creek. Although it does not show in the 1998 aerial photo base map (Figure 23), a rural residential site has been developed in the parcel directly north of the BLM parcel where sample site 22 is located. It is possible that contaminated nursery stock planted in this area could have served as the original source of the pathogen in the area.

The presence of this additional pathogen within the range of *A. myrtifolia* has the potential to further complicate disease management. Pathogenicity tests are still needed to confirm that *P. cambivora* is pathogenic to *A. myrtifolia* and *A. viscida,* Although pathogenicity appears likely, based on the association of *P. cambivora* with dead and dying manzanitas, it will be important to determine whether *P. cambivora* is as virulent as *P. cinnamomi*. If it is not, overall plant- and stand-level symptoms associated with this root pathogen may be less distinct than those associated with *P. cinnamomi*. *P. cambivora* could also exhibit different levels of pathogenicity to hosts other than *Arctostaphlyos* spp., which could result in differences in pathogen survival and spread relative to *P. cinnamomi*. Nonetheless, the same overall strategies recommended to prevent the spread of *P. cinnamomi* should also be effective against *P. cambivora*.

Because *P. cambivora* was not detected in any other sampling sites, it does not appear to be widespread at present. It is possible that *P. cambivora* was only recently introduced to the area and/or it may be less adapted to the local environment than *P. cinnamomi*. Given the information gaps that exist for this species, we strongly recommend that efforts be made to delimit the extent of this infestation and restrict future spread of this pathogen.

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