Management of diseases caused by *Phytophthora* on the SFPUC Peninsula Watershed

Parts 1 and 2: Sudden oak death management studies

Final project report for Forest Service Agreement No. 12-JV-11272131-054

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Cover images: Clockwise from upper left - Portion of tanoak stand in phosphite-sudden oak prevention (SOD) study; Elizabeth Bernhardt collecting a water sample for *Phytophthora* testing from Crystal Springs Reservoir; untreated control coast live oak killed by SOD; nursery bench leachate test for detecting *Phytophthora*-infected stock.

Executive summary

We studied management methods for preventing sudden oak death (SOD), caused by *Phytophthora ramorum*, in large field studies on the SFPUC Peninsula Watershed. Removal of California bay (*Umbellularia californica*) around coast live oaks (*Quercus agrifolia*), was effective at preventing new disease development over the 7-year study period. SOD developed in 25% of the untreated control trees but no SOD was observed in the stand treated by area-wide bays removal.

We also studied the use of potassium phosphite for preventing SOD in tanoak. Phosphite was applied as a trunk spray at the product label rate (22.36% a.i. aqueous solution + Pentra-Bark® surfactant at 2.3% v/v) to a 1.35 ha block of 233 large-diameter tanoaks (*Notholithocarpus densiflorus*). Annual phosphite applications began in 2008; symptoms of *P. ramorum* were not seen in the stand until 2011. In 2013, SOD incidence in treated trees was 32% compared to 18% in adjacent untreated trees. Subsequent discontinuation of phosphite treatment did not affect disease progress; SOD continued to increase at similar rates in phosphite-treated and control trees, reaching 47% among phosphite-treated trunks and 32% in untreated trunks by 2016. Preventative phosphite application did not delay SOD onset or reduce SOD incidence or SOD-related mortality.

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Introduction: Sudden oak death studies

Since its introduction into California in the mid 1990s, *Phytophthora ramorum* is estimated to have killed millions of trees (Cunniffe et al. 2016). In California forests, *P. ramorum* functions primarily as an aerial pathogen, sporulating on leaves or twigs of various hosts, but causing lethal bole cankers only on a few species, including tanoaks (*Notholithocarpus densiflorus*), coast live oaks (*Quercus agrifolia*), California black oaks (*Q. kelloggii*), canyon live oaks (*Q. chrysolepis*), and Shreve oaks (*Q. parvula* var. *shrevei*) (Rizzo et al. 2002, Swiecki et al. 2016).

Two large-scale studies were established to prevent SOD in susceptible tanoaks and coast live oaks on the Peninsula Watershed in 2008 and 2009, respectively. In this report we summarize results for these studies through 2016.

1. Protection of coast live oak from SOD by removing nearby California bay

1.1. Introduction

SOD in susceptible oak species is largely a byproduct of the *P. ramorum* foliar disease cycle on California bay. When conditions are favorable for foliar disease development in California bay (*Umbellularia californica*), large numbers of spores from infected leaves are dispersed to nearby oaks by dripping and splashing water. *P. ramorum* only produces spores on twigs and leaves of oaks under very wet conditions when inoculum levels from California bay are already very high, so additional inoculum produced on oaks is not epidemiologically important. SOD incidence, severity, and mortality rates increase as the distance from oak trunk to California bay foliage decreases (Swiecki and Bernhardt 2002, 2008). Coast live oaks with California bay foliage directly over or within 1.5 m of the trunk have the highest risk of infection and mortality. Disease risk also increases as the total amount of California bay cover within 2.5 to 5m of the oak trunk increases (Swiecki and Bernhardt 2008).

Foresters have long manipulated stands to favor one species over another. The spatial relationship between California bay and SOD in susceptible oak species suggests that removing California bay from the vicinity of susceptible oak trees should lessen disease pressure and provide a means for preventing infection of oaks by *P. ramorum*. Disease in tanoak is also greatly increased in the presence of California bay (Davidson et al. 2005), but the fact that *P. ramorum* causes sporulating foliar and twig infections in tanoak rules out California bay removal as an effective long-term means of preventing SOD in tanoak.

California bay removal can be implemented at various scales (Swiecki and Bernhardt 2013a). Area-wide removal of California bay from entire stands of oaks is likely to reduce inoculum near oaks to the greatest degree and should be the most effective treatment for preventing SOD development and mortality in susceptible oak species. This approach was tested on coast live oaks near the Pulgas Water Temple.

1.2. Methods

1.2.1. Study design

In 2009 we set up a prospective cohort study to investigate the effect of California bay (*Umbellularia californica*) removal as a method for preventing SOD. Prior to applying the bay-removal treatment, control and treated trees selected for monitoring were rated as being at moderate to high risk of *P. ramorum* infection. Risk assessment was based on proximity to bay and oak-related risk factors. Disease status was assessed before the bays were removed. Reported analyses consider only trunks that were free of SOD symptoms at the study baseline date.

1.2.2. Study location

The Pulgas Water Temple receives many visitors as a tourist stop and popular outdoor wedding venue. Two areas with large mature coast live oaks near the Pulgas Water Temple were selected for area-wide bay removal (Figure 1-1). In both areas, California bay was dispersed throughout the understory as numerous saplings and a few small trees. Area-wide bay removal was conducted in a stand (0.5 ha) along a seasonal creek directly adjacent to the Pulgas Temple parking area and in a 0.4 ha section of a stand on a hillside about 200 m north of the first stand. Both stands are fenced to prevent public access. Within the stand adjacent to the parking area, one coast live oak had been removed prior to the start of our study; we could not confirm whether it was killed by SOD. Mixed coast live oak-California bay stands with documented SOD mortality were located within 1 km of the treated area.

In September 2009, we tagged 77 coast live oaks (84 trunks) within the treatment areas for monitoring. Individual trunks of multitrunked oaks commonly have different disease outcomes (Swiecki and Bernhardt 2013a) and were monitored separately. Several trees had bleeding cankers, but isolations from cankers were negative for *P. ramorum* and other *Phytophthora* spp. Some of the observed bleeding was associated with sycamore borer infestations in the bark of the lower trunks. California bays were removed from the treated areas in October 2009, with follow up work completed by January 2010. All felled material was removed from the treated areas and chipped.

We selected control trees from the nearest available area, a coast live oak-dominated stand with variable California bay cover, about 1 km west of the treated stands. In this area, many of the oaks already had SOD symptoms in areas with dense bay cover. We located and tagged 48 uninfected control trees (59 trunks), mostly around scattered individual bay trees (Figure 1-1). GPS coordinates were recorded for all tagged study trees.

Baseline oak health data were collected at the start of the study and at one to two year intervals thereafter. The presence of bleeding trunk cankers was noted and their locations were recorded using cardinal directions and height above ground. The percent of trunk circumference affected by cankers was estimated using a 0-6 scale: 0 = not seen; 1= up to 2.5%; 2 = 2.5-19%; 3 = 20-49%; 4 = 50-79%; 5 = 80-97.4%; 6 = 97.5-100%. The scale is pre-transformed using the arcsine transformation (Little and Hills 1978). At each annual evaluation, new suspect cankers were identified by using a hatchet to chip away small

areas of outer bark to expose the canker edge. Suspect SOD cankers were sampled for the presence of *P. ramorum* by culturing small tissue pieces from the canker margin on PARP medium (Erwin and Ribeiro 1995) in petri plates. The percent of trunk circumference colonized by beetles or showing sporulation of *Annulohypoxylon thouarsianum,* and percent canopy dieback were also scored using the 0-6 scale above. Tree decline or mortality due to factors other than *P. ramorum,* trunk and root failures, and any other relevant symptoms were also recorded at each evaluation.

Figure 1-1. Overview of bay removal SOD management zone at Pulgas Water Temple and control trees in nontreated areas. Disease status as of December 2016 is represented. Blue dots represent tagged coast live oaks in bay removal zones (all were asymptomatic). White circles represent asymptomatic control trees. Magenta circles represent control trees with SOD symptoms as of January 2017. Bay removal areas are bounded by orange lines.

Variables describing California bay distribution and density around each oak trunk were recorded at the start of the study and at each subsequent evaluation. For treated oak trunks, measurements were made before and after bay removal. We used a 500mW green laser attached to an angle gauge to project a plumb line to the edge of bay canopy nearest to each oak trunk. A laser rangefinder was used to measure the horizontal distance from this vertical line to the oak trunk (bay foliage-oak trunk distance). We visually estimated the bay canopy cover for zones within 2.5 and 5m of each oak trunk using a modified quarter scale: 0 = 0% cover, 0.1 = trace amounts of cover (<1%), 1 = 1-25% cover, 2 = 26-50% cover, 3 = 51-75% cover, and 4 = more than 75% bay cover. We also noted whether overstory or understory bay trees were present within 10 and 20 m of the oak trunk and if bay seedlings were located within 1 m of the oak trunk.

1.2.3. Evaluation dates

We recorded initial baseline data on trees in the bay removal area in September 2009, prior to bay removal. Trees were tagged and tree diameter, disease status, and bay neighborhood data was recorded. Controls were identified, tagged, and rated in early 2010. All study trees were reevaluated between August and November 2011, between 31 August 2012 and 15 February 2013, and between 18 and 21 September 2015. We last re-evaluated the coast live oak controls in this experiment on 2 Dec 2016. Treated trees were evaluated 6 January 2017.

1.2.4. Data analysis

Exact binomial confidence intervals (Clopper and Pearson 1934) were plotted to compare the proportions of symptomatic trees between treatments.

1.3. Results

Through January 2017, no SOD infections were observed among the monitored coast live oaks in bay removal areas. In the control cohort, SOD symptom incidence has increased gradually (Figure 1-2). By December 2016, about 25% (15/59) of the untreated control trees had developed SOD symptoms and ten of these trees (17% of controls) had been killed by SOD. Initial bay foliage-oak trunk clearances of the SOD-affected control trees ranged from 0 to 2.5m.

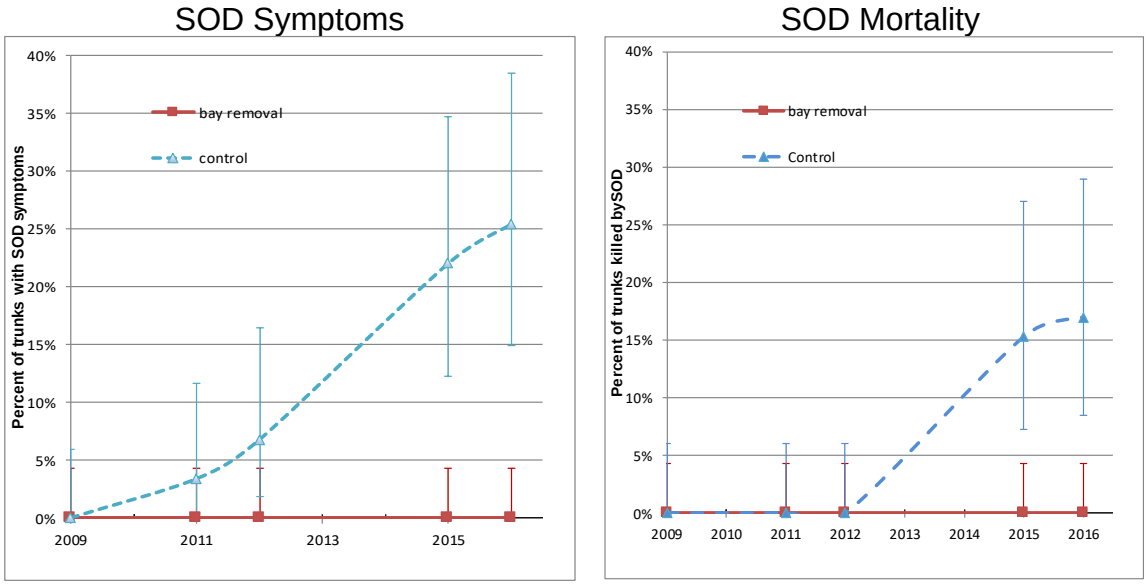

Figure 1-2. Percent of coast live oak study trees with SOD symptoms over time by management treatment (California bay removal or not). Data from 6 Jan 2017 is graphed with the December 2016 data. Error bars represent 95% exact binomial confidence intervals.

Resprouting has occurred from the cut stumps of the removed California bay, and a few small bays along the fence line with the Pulgas parking lot were missed during initial bay removal treatments. We have periodically removed sprouts from stumps of bays near monitored trees and cut a few missed saplings during tree health monitoring. Deer browsing has also limited bay sprout regrowth in much of the treated area, but less accessible sprouts have not been browsed substantially. Additional maintenance to

remove bay sprouts was clearly needed within the bay removal area by late 2016, seven years after the trees were originally removed.

Although cut stumps were reported to have been treated with glyphosate at the time of California bay removal, the trees may have been too water-stressed in October 2009 for effective translocation of the herbicide. We have seen in other locations that glyphosate stump treatments of California bay are most successful in January through March (Swiecki and Bernhardt 2013a). Low efficacy of the glyphosate treatment may also have been associated with delays between cutting and glyphosate application to the stump or use of a glyphosate rate that was too low. Furthermore, many of the bay stumps were small in diameter, and we have previously observed that glyphosate efficacy on cut stumps is typically poorer in small diameter stumps than in larger ones.

1.4. Discussion

Based on the epidemiology of SOD in susceptible oaks, it is not surprising that removing California bay near susceptible oaks greatly reduced their risk of developing SOD symptoms. Variables describing the proximity and density of California bay in the local oak neighborhood are the strongest predictors of SOD development in coast live oak in California forests (Swiecki and Bernhardt 2015). *P. ramorum* infects bay foliage and sporulates abundantly on it, but does not sporulate on coast live oak cankers (Davidson et al. 2002, 2005). The amount of *P. ramorum* inoculum dispersed from bay canopies decreases strongly as the distance from the bay canopy source increases from 0 to 5m or beyond (Davidson et al. 2005).

Where practical and consistent with other management objectives, area-wide removal of California bay appears to provide the greatest level of SOD prevention for stands of susceptible oaks (Swiecki and Bernhardt 2013a). Area-wide bay removal maximizes the distance between inoculum sources and susceptible oaks, thereby minimizing exposure to inoculum. This study and a similar study with Shreve oak (Swiecki and Bernhardt, submitted) showed that area-wide bay removal completely suppressed disease over a 7-year period. However, several drought years, which are unfavorable for *P. ramorum* reproduction, occurred during the study period. Continued monitoring of the bay removal plots is needed to determine long-term efficacy under weather conditions that generate very high inoculum levels.

Responses of oaks to *P. ramorum* vary greatly, with many trees expressing resistant or tolerant reactions to infection (Swiecki and Bernhardt 2013a). Consequently, some oaks with high local California bay cover may resist infection, whereas highly susceptible individual oaks sometimes become infected where bay foliage-oak trunk clearances are greater than 10 to 20 m. In such situations, infections appear to be initiated by small numbers of spores, either blown by wind or vectored by animals or human activities.

While bay removal may not protect all susceptible individual oaks, it has the potential to greatly reduce or largely eliminate SOD impacts in a variety of situations. Additional use of this management technique on the Peninsula Watershed could protect stands of oaks from SOD mortality and reduce associated issues related to tree failure hazards, fuels

accumulations, and invasive plant spread in SOD affected areas. We previously identified a number of locations near roads on the Peninsula watershed where the balance between coast live oak canopy and bay populations is favorable for implementing area-wide bay removal.

2. Managing SOD in large tanoaks with potassium phosphite

2.1. Introduction

In general, tanoak is more susceptible to *P. ramorum* than are SOD oak hosts (Rizzo et al. 2002, Swiecki and Bernhardt 2013a). Furthermore, *Phytophthora ramorum* sporulates readily on tanoak twig cankers (Davidson et al. 2008). Sporangia and zoospores can be dispersed by windblown rain to other parts of the canopy, initiating additional leaf and twig infections. Spores can also be splashed from infected tanoak twigs to canopies of adjacent tanoaks, so *P. ramorum* can readily spread between trees in a tanoak stand. This is different than the disease epidemiology in coast live oaks, where SOD is a byproduct of foliar infections on California bay.

Search for control methods began as soon as sudden oak death (SOD) was recognized as a new disease (Swiecki and Bernhardt 2016a). Treating stems of infected trees with potassium phosphite as a spray application was identified as a possible chemical control method (Garbelotto at al. 2007, Garbelotto and Schmidt 2009). Various assays have shown that phosphite can reduce the rate of *P. ramorum* lesion expansion in treated trees (Garbelotto and Schmidt 2009, Garbelotto et al. 2007).

Potassium phosphite (also known as potassium phosphonate, or mono- and di-potassium salts of phosphorous acid) is a selective, systemic chemical that has been used to manage *Phytophthora* diseases, particularly root rot caused by *Phytophthora cinnamomi* (Guest and Grant 1991). The exact mechanism by which phosphite controls *Phytophthora* is not totally understood. Concentrations in a plant may be high enough to be directly toxic to *Phytophthora* in some cases. At lower concentrations, phosphite stimulates the plant to mount a resistant reaction in response to infection (Guest and Grant 1991, Hardy et al 2001). Phosphites have a high level of environmental safety and very low nontarget toxicity. EPA classifies phosphites as biopesticides because these salts are closely related to common, widely occurring substances (USEPA 1998).

Although potassium phosphite pesticides (e.g., Reliant®, Agri-Fos®) list bark spray application for control of *P. ramorum* on their labels, no large-scale field tests of this treatment in tanoak had been conducted before our study was initiated. Because trunk spray applications of potassium phosphite to large numbers of trees entails substantial ongoing cost and effort, the treatment is most likely to be used to protect high-value trees or stands from SOD. Furthermore, treatment efficacy needs to be high to justify its recurring costs for repeated applications. An effective treatment would maintain the number of SOD killed trees to a low level indefinitely (minimize the final size of the outbreak) and should delay onset of SOD in a population that is exposed to *P. ramorum* inoculum.

When applied to tanoak, phosphite application could prevent disease by one or both of the following mechanisms: (1) suppressing foliar and twig infections to reduce local inoculum production and (2) increasing the tree's resistance to bole infections. If the first mechanism is important, efficacy of phosphite should be maximized by treating all tanoaks in a large contiguous area. Small plots or individual tree treatments are unlikely to allow for expression of reduced inoculum production effects because inoculum from adjacent non-treated trees could be splashed and blown onto treated trees.

To take advantage of potential reduction in local inoculum production, all phosphite-treated trees in this study were in a large contiguous area. In addition, phosphite treatments were initiated well before the tanoak population was exposed to *P. ramorum* inoculum. Nontreated tanoaks located beyond the perimeter of the phosphite-treated population were monitored to assess when *P. ramorum* infections appeared and how the spatial distribution of SOD developed across the study area over time. Exposure of individual trees in tanoak stands to *P. ramorum* cannot be controlled experimentally, is spatially nonuniform, and varies over time. However, by monitoring a large population of trees in a contiguous area over a long period of time, it is possible to detect a strong treatment effect after the area has been invaded by *P. ramorum.*

2.2. Methods

2.2.1. Study location and design

The study location is on the SFPUC Peninsula Watershed lands on a ridge southwest of Crystal Springs Reservoir. The study area is on a flat ridge 10 km west of the Pacific Ocean where summer fog intrusion is common, near the edge of the watershed property. This area contained the largest stand of mature tanoak on the watershed and had not yet been affected by SOD when the study was undertaken. SFPUC resource managers had identified this as a unique, high-value stand, consisting of mostly large diameter tanoaks (range 6.5cm to 93 cm, mean 46 cm). Most of the tanoaks were at least 30 m tall, with long, clear trunks. The tanoaks were intermixed with large second growth coast redwoods (*Sequoia sempervirens*) and some madrone (*Arbutus menziesii*). The stand appears to have developed after the area was logged in the late 19th or early 20th century. Canopy cover was nearly complete within the study area.

The entire study area was similar in stand composition and structure and topographic position. No *a priori* factors were identified to indicate that SOD risk would vary across the study area. The treated trees were within a roughly rectangular area, about 170 m long and 60 to 100 m wide (about 1.35 ha total) that included 233 tanoak trunks from both single- and multi-trunked trees (average tanoak density 173 trunks/ha). Control trees were in four areas distributed around the treated trees (Figure 2-1). We monitored about 60 trunks in each control area, which were separated from the treated trees by a buffer of at least two tree canopy widths. The four control areas (1.37 ha in aggregate) had a total of 243 monitored trunks (average tanoak density 177 trunks/ha). A single small (DBH about 12 cm) California bay with no *P. ramorum* foliar symptoms located within the phosphite treated area was removed prior to the start of the study. No other California bay were located within at least 100 m of the study area. At the start of the study in 2008, no evidence of SOD was observed within 1 km of the stand, but SOD-

killed coast live oaks were present 2.5 km east of the stand. Some scattered tanoak mortality seen in the study area was related to root disease, mostly associated with *Armillaria gallica*.

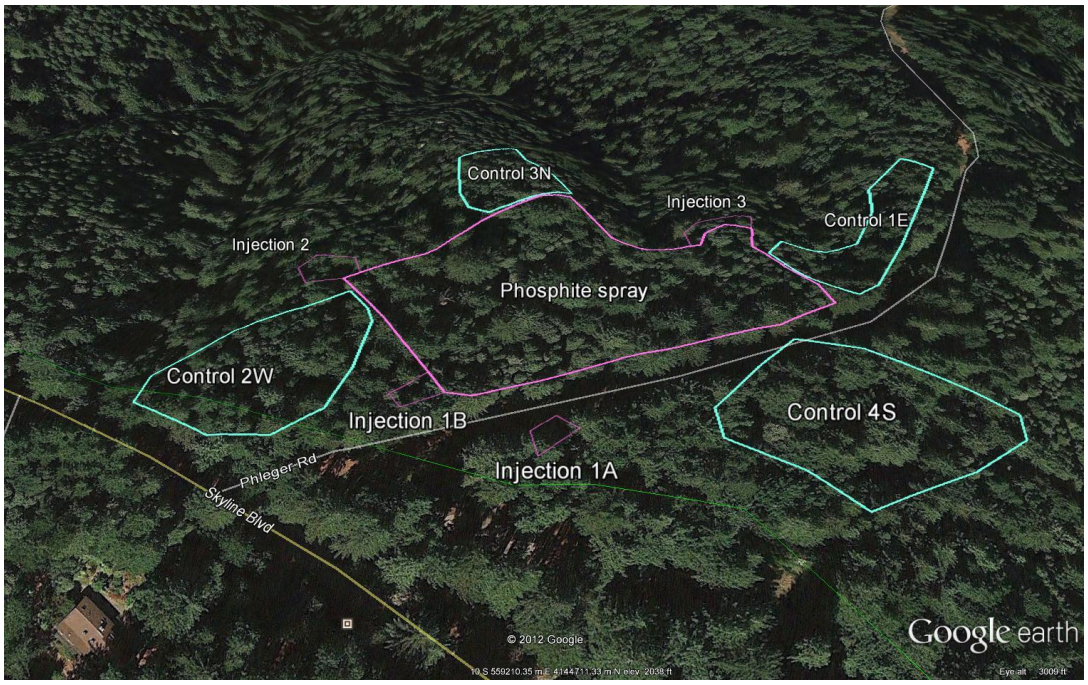

Figure 2-1. Aerial image of plot area with approximate plot boundaries superimposed (magenta outlines=phosphite treated areas, cyan outlines = control areas). The road that continues as the southern firebreak is listed as Pfleger Road in the image. Control plot 4S extends onto the Phleger Estate. Image date April 2012. Some tanoaks were treated by stem injection (light magenta areas). All injected trees in injection plot 1A were removed for reasons unrelated to the study in 2012. Data on the phosphite-injected trees were previously reported (Swiecki and Bernhardt 2013b) and are not discussed here.

2.2.2. Tree observations and data

At the start of the study, numbered aluminum tree tags were applied and DBH (diameter at 1.37m above grade) and baseline tree health data (described below) were recorded. We recorded the percentage of each tanoak canopy that was surrounded by other tanoak canopies in 25% increments (0=none, 1=1-25%, 2=26-50%, 3=51-75%, 4=76-100%). We used the same rating sytem to quantify the amount of canopy surrounded by phosphite-treated tanoaks, to account for potentially lower inoculum production from adjacent treated trees. The presence of adjacent redwood and madrone canopy was recorded as a binary factor (yes/no). We estimated the amount of moss present in the area of the trunk to be sprayed prior to the first phosphite application using a 0-6 scale: 0 = not seen; 1= up to 2.5%; 2 = 2.5-19%; 3 = 20-49%; 4 = 50-79%; 5 = 80-97.4%; 6 = 97.5-100%. The scale is pre-transformed using the arcsine transformation (Little and Hills 1978).

Tree condition was assessed at the start of the study and annually thereafter in the late summer or fall. The presence of bleeding trunk cankers was noted and their locations were recorded using cardinal directions and height above ground. The percent of trunk circumference affected by cankers was estimated using the pre-transformed 0-6 scale described above. At each annual evaluation, new suspect cankers were identified by

using a hatchet to chip away small areas of outer bark to expose the canker edge. Suspect SOD cankers were sampled for the presence of *P. ramorum* by culturing small tissue pieces from the canker margin on PARP medium (Erwin and Ribeiro 1995) in petri plates. The percent of trunk circumference colonized by beetles or showing sporulation of *Annulohypoxylon thouarsianum*, and percent canopy dieback were also scored using the 0-6 scale above. Tree decline or mortality due to factors other than *P. ramorum*, trunk and root failures, and any other relevant symptoms were also recorded at each evaluation. In 2012, all tree locations were mapped using a combination of GPS coordinates and distance-azimuth measurements between trees, collected with a Criterion 400 survey laser (Laser Technology, Inc.).

Prior to the start of treatments, we collected 20 soil samples from throughout the area where trees were to be treated with phosphite. Elizabeth Fichtner (D. Rizzo lab, UC Davis) used rhododendron leaf disks to test the samples for the presence of *P. ramorum* and other *Phytophthora* species (Fichtner et al. 2007).

2.2.3. Phosphite treatments

From 2008 through 2011, phosphite was applied at the product label rate: i.e., a 22.36% a.i. aqueous solution. In 2012, the applied solution concentration was increased to 29.8% a.i. In all years, Pentra-Bark® surfactant was added to the spray mix at the 2.3% v/v rate specified on the product label. Measured trunk diameters were used to calculate the amount of phosphite solution to apply to each trunk using methods described previously (Swiecki and Bernhardt 2007). Trees up to 30.5 cm DBH received 31 ml spray solution/cm DBH. For trunks larger than 30.5 cm DBH, the applied volume was calculated as follows:

$$\text{total spray vol, L} = -6.641803 + 0.1454801 \times (\text{DBH, cm}) + 0.0005723 \times ([\text{DBH, cm}]-104.14)^2$$

This formula increases the dose for large diameter trees so that the applied volume remains more closely proportional to bark/sapwood volume. The phosphite spray dose was applied to each trunk by calculating the time that each trunk needed to be sprayed based on the calculated spray amount and spray head delivery rate. The largest tanoak trunk (93 cm DBH) received 6.97 L of spray solution (75 ml/cm DBH) and required 3 minutes and 49 seconds of spray application time at the 30.4 ml/sec delivery rate of the spray head.

The initial application required about 477 L of spray solution to spray the 233 trunks. Phosphite was applied by Mayne Tree Expert Company (San Carlos, CA) using a 95 L spray tank with a 12 VDC electric pump. A digital motor speed controller was used to modulate the output of the sprayer. The sprayer head consisted of two TeeJet AI11003VS air induction nozzles oriented vertically so the long axis of the fan-shaped pattern was oriented along the trunk axis. The nozzles were mounted about 18 cm apart on a vertical frame and the sprayer head was mounted on a telescoping pole. To favor absorption through the thinner bark and maximize potential for absorption as residues were remobilized by rainwater, and the spray was banded on the trunk starting at a height of about 6 m and working downward, typically at least 2 to 3 m (Figure 2-2). Sprayer calibration was checked at the start and periodically during each day of application by

collecting and measuring the volume of solution delivered in 20 seconds. A pressure gauge at the base of the spray pole was monitored to assure that the sprayer remained in calibration. Total application volume was monitored by auditing the amount of material mixed and the amount of spray solution left over after each application and was typically within 5% of the target volume. Phosphite applications took 3 to 4 days by the 3-person crew and were completed within a one week period. November applications were scheduled to occur after the first autumn rains. Initial phosphite applications were made in May/June and November 2008. Annual applications were subsequently completed in November from 2009 through 2012, after which phosphite applications were discontinued.

Figure 2-2. Phosphite trunk spray being applied high on the trunk of a mature tanoak in the SOD prevention study on the San Francisco Peninsula.

2.2.4. Data analysis

Analyses of the tanoak phosphite study are complicated by possible but unknown non-independence of experimental units: because *P. ramorum* inoculum is produced on tanoaks, disease outcomes could be influenced by neighboring trees to differing degrees within the treated and control populations. Logistic regression analyses to examine tree and plot factors related to disease outcomes were therefore conducted within treated and control populations separately using JMP statistical software, SAS Institute Inc., Cary, NC. Exact binomial confidence intervals (Clopper and Pearson 1934) were plotted to compare the proportions of symptomatic trees between treatments.

2.3. Results

2.3.1. SOD incidence and SOD-related mortality

No *Phytophthora* species were detected in plots in the 2008 soil samples and no evidence of *Phytophthora ramorum* was observed in the plot area until twig blighting of understory tanoak was first observed in spring 2011 in a control area. By fall 2011, twig

blighting was widespread and *P. ramorum* was isolated from understory tanoak twigs and leaves throughout the study area, including the phosphite-sprayed plot. At this time, some tanoaks in both treated and control plots also showed foliage blighting consistent with *P. ramorum* symptoms in their high canopies. No *P. ramorum* trunk cankers were observed on tanoaks until fall 2012, when cankers were observed in treated and control areas (Figure 2-3). Several trees had nearly complete canopy dieback in April 2012.

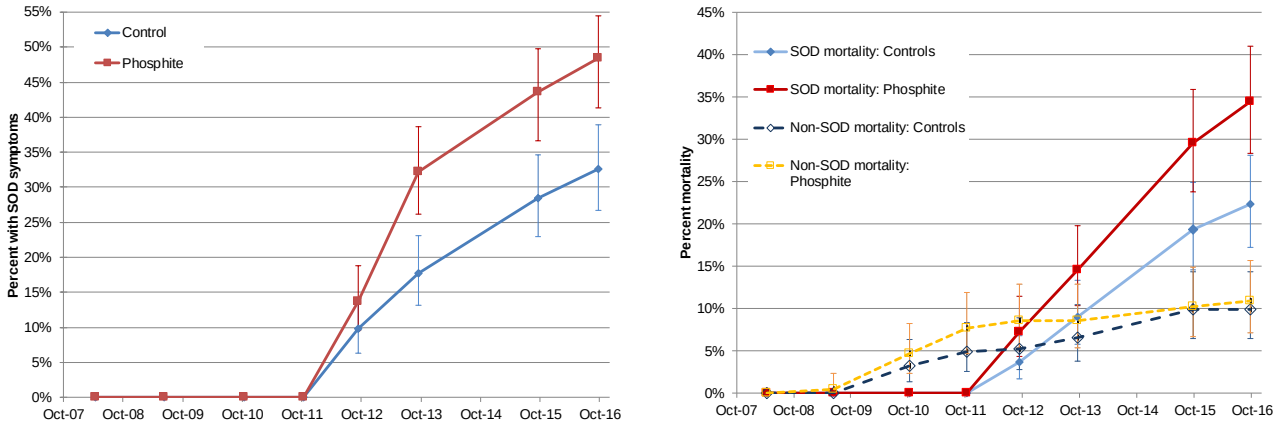

Figure 2-3. Incidence of SOD trunk symptoms (left) and mortality due to SOD and other causes (right) in mature tanoaks either treated annually with potassium phosphite from May 2008 through November 2012 (phosphite) or left untreated (controls). *Phytophthora ramorum* foliar symptoms first appeared in the study populations in 2011. Error bars are 95% exact binomial confidence intervals.

Initially, very few of the symptomatic tanoaks showed bleeding associated with *P. ramorum* cankers. Over time, the incidence of bleeding cankers in symptomatic trees increased, but was still below 50% overall in 2016 among trees showing late SOD symptoms (secondary attack by beetles and or *Annulohypoxylon thouarsianum*). Many affected trees developed high bole cankers and had dead tops even though we could not detect cankers in the lower 2.5 m of the trunk. We used binoculars to scan the upper portions of the boles for higher bleeding cankers, but did not see evidence of bleeding in the upper boles of most trees. In some trees with dead canopies and no external bole symptoms, chipping of the bark revealed cankers with a bottom edge 1.5 m or more above the soil level. We were able to verify one high bole canker (starting at a height of 9.6 m) in a tree that was cut down in May 2013. Positive isolations of *P. ramorum* were obtained from most of the bark cankers we sampled.

As shown in Figure 2-3, SOD symptoms and SOD-related mortality appeared in similar proportions of phosphite-treated and control tanoaks in fall 2012, indicating that phosphite application did not delay the onset of the epidemic within the treated population. Given this apparent lack of efficacy after 4 years of preventative phosphite application at the standard application rate (22.4% solution), we applied a higher phosphite dose (29.8% solution) to study trees in fall 2012. By fall 2013, one year following this final phosphite application, the proportion of tanoak trunks with SOD symptoms was significantly higher in the phosphite-treated population than the controls (Figure 2-3).

Cessation of phosphite treatments after 2012 had no apparent effect on the increase in SOD incidence or mortality over time in the treated trees compared to the controls, based on fall 2015 and 2016 evaluations (Figure 2-3). This is a further indication that the phosphite treatment had no effect on disease. In 2016, both SOD incidence and SOD-related mortality were significantly higher among phosphite-treated trees than controls (Figure 2-3).

The distribution of SOD-affected trees in the study area was nonuniform with evident spatial clustering (Figures 2-4, 2-5, 2-6). Logistic regression models within treated and control populations for 2013 binary disease outcomes (SOD symptoms and SOD-related mortality) failed to identify any significant tree-related predictors. Trunk DBH, the amount of adjacent tanoak canopy, and presence of adjacent redwood or madrone canopy, were not significant predictors of SOD or SOD-related mortality. The degree to which phosphite-treated tanoaks were surrounded by other phosphite-treated tanoaks was also unrelated to disease outcomes, suggesting that inoculum production on tanoak twigs and foliage was not substantially suppressed by phosphite application. Moss cover on treated trunks was also not related to SOD outcomes, indicating that lack of efficacy was not due to absorption of spray by moss. Moss on trunks was killed by the initial phosphite application and subsequently degraded. Any absorption of spray by moss would have been limited to the first year of treatment, several years before the pathogen was detected in the study area.

Prior to the appearance of SOD-related mortality in 2012, scattered mortality due to Armillaria root disease, was observed in the study area (Figure 2-3). Over the 8.5 years of observation, mortality due to factors other than SOD was 10.4% overall (average 1.2% per year), and was nearly identical in phosphite-treated and control trees (Figure 2-3). By December 2016, SOD-related mortality across control and treated populations (28.2% overall) significantly exceeded background mortality due to other factors (Figure 2-3). SOD-related mortality averaged 7.1% per year between 2012 and 2016.

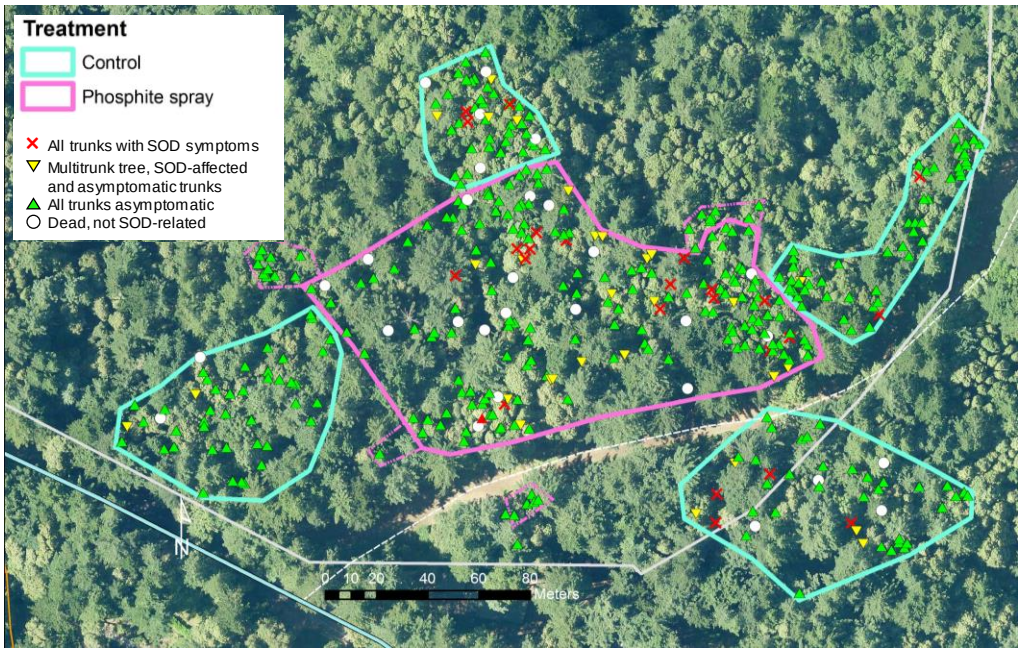

Figure 2-4. Distribution of tanoaks with SOD symptoms or non-SOD mortality in fall 2012 in tanoaks treated with potassium phosphite trunk applications (from May 2008 through November 2012) or left untreated (control). Photo date 2011.

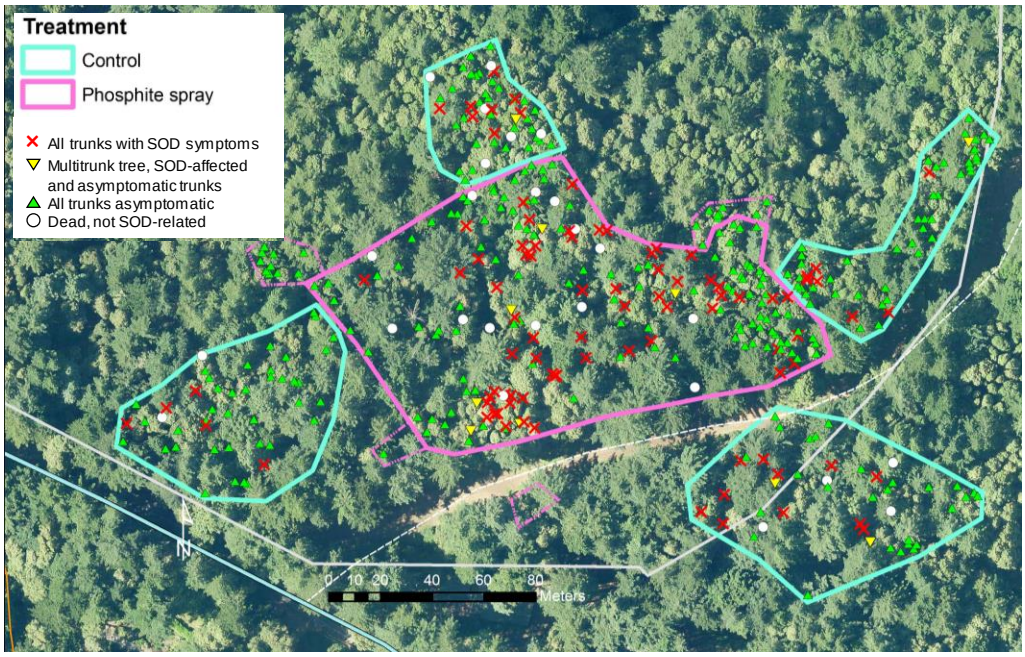

Figure 2-5. Distribution of tanoaks with SOD symptoms or non-SOD mortality in fall 2013, in tanoaks treated with potassium phosphite trunk applications (from May 2008 through November 2012) or left untreated (control). Photo date 2011.

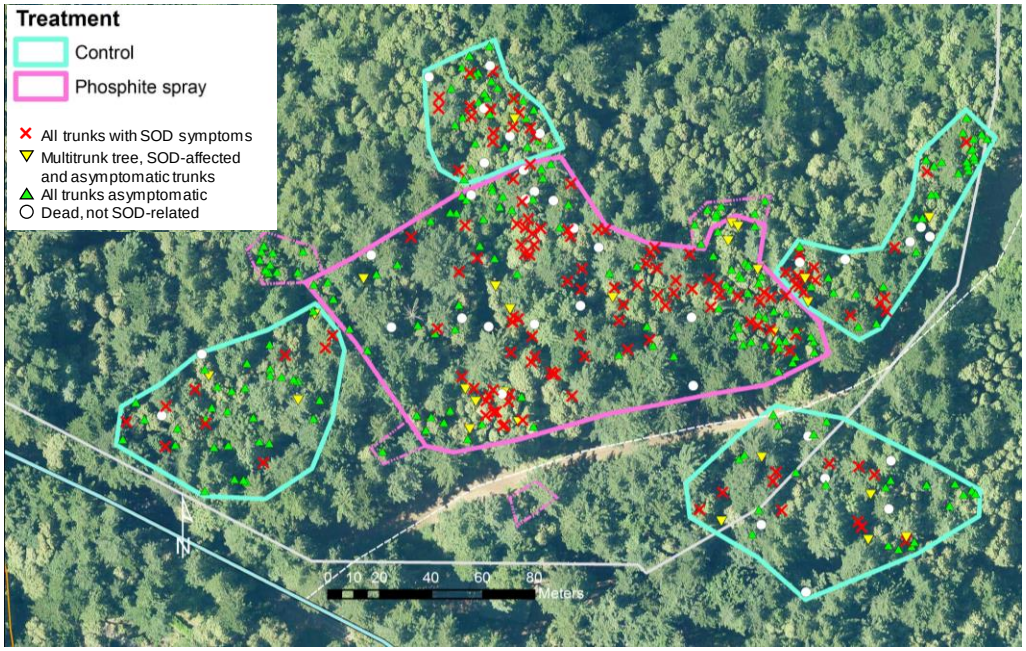

Figure 2-6. Distribution of tanoaks with SOD symptoms or non-SOD mortality in fall 2016 in tanoaks treated with potassium phosphite trunk applications (from May 2008 through November 2012) or left untreated (control). Photo date 2011.

2.3.2. Tree failure

Only a few failures were seen among tanoaks prior to the movement of SOD into the study area in 2011. These failures were in trees affected by Armillaria root disease and/or canker rots caused by wood decay fungi. Since *P. ramorum* entered the stand, an increasing number of trees have died due to SOD, however failure rates are still relatively low among these SOD-killed trees (Figure 2-7, Figure 2-8).

We recorded height above ground of all bole, root, and root crown failures and branch/scaffold failures greater than 20 cm diameter at the break. Only 3 of the bole failures were less than 20 cm, and 2 of these occurred in small trees with DBH less than 20 cm. Through September 2016, 93% of recorded failures were bole failures; the remainder were root or root crown failures.

Most of the tanoaks in the stand were initially sound, with little preexisting wood decay. Among the first SOD-killed cohort in 2012, failures were first recorded in 2015 (Figure 2-7). Since that time, more failures have occurred in shorter intervals since recorded tree mortality. The pattern suggests that many of the SOD-affected trees dying after 2012 may have been infected at the same time as those dying in 2012. This is supported by the progression of disease symptoms within the study area over time (Figure 2-8), which is consistent with an initial pulse of infection with few new infections during the following drought years.

The fact that the tanoak boles were initially sound before SOD infection may explain why a relatively large percentage of standing dead trees have not yet failed (Figure 2-7, bottom), especially compared to tanoaks killed by other agents (Figure 2-7, top). In addition, most of the tanoaks in this stand have no low branches, with all branching

occurring in the upper canopy in an excurrent pattern. As a result, there are relatively few large-diameter scaffold and branches (Figure 2-9). Although small branch failures (<20 cm) have occurred in the SOD and *Armillaria*-killed trees in the stand, recorded failures with diameters of 20 cm or more are all bole failures.

Many of the bole failures to date are high above the ground where diameters are smaller and will be more rapidly weakened by borers and sapwood decay caused by *A. thouarsianum*. The upper stems are also more likely to experience greater stresses associated with wind. The mean height of bole failures of SOD-killed trees was 12.5 m compared to 4.7 m for trees killed by *Armillaria* and other causes (two tailed *t* test P <0.0001, N=60 trees). This likely reflects the fact that *Armillaria* and other agents killing trees are wood decay fungi and cause loss in wood strength at the base as well as other areas in the lower bole. In addition, mortality associated with *Armillaria* is more likely to affect smaller and more suppressed trees. The mean DBH of SOD-killed tanoaks was 45.1 cm compared with a mean DBH of 28.4 cm for those killed by other agents (two tailed t test P<0.0001, N=184 trees). Nonetheless, we have observed a few large-diameter trees (40-74 cm DBH) killed by *Armillaria* in this stand.

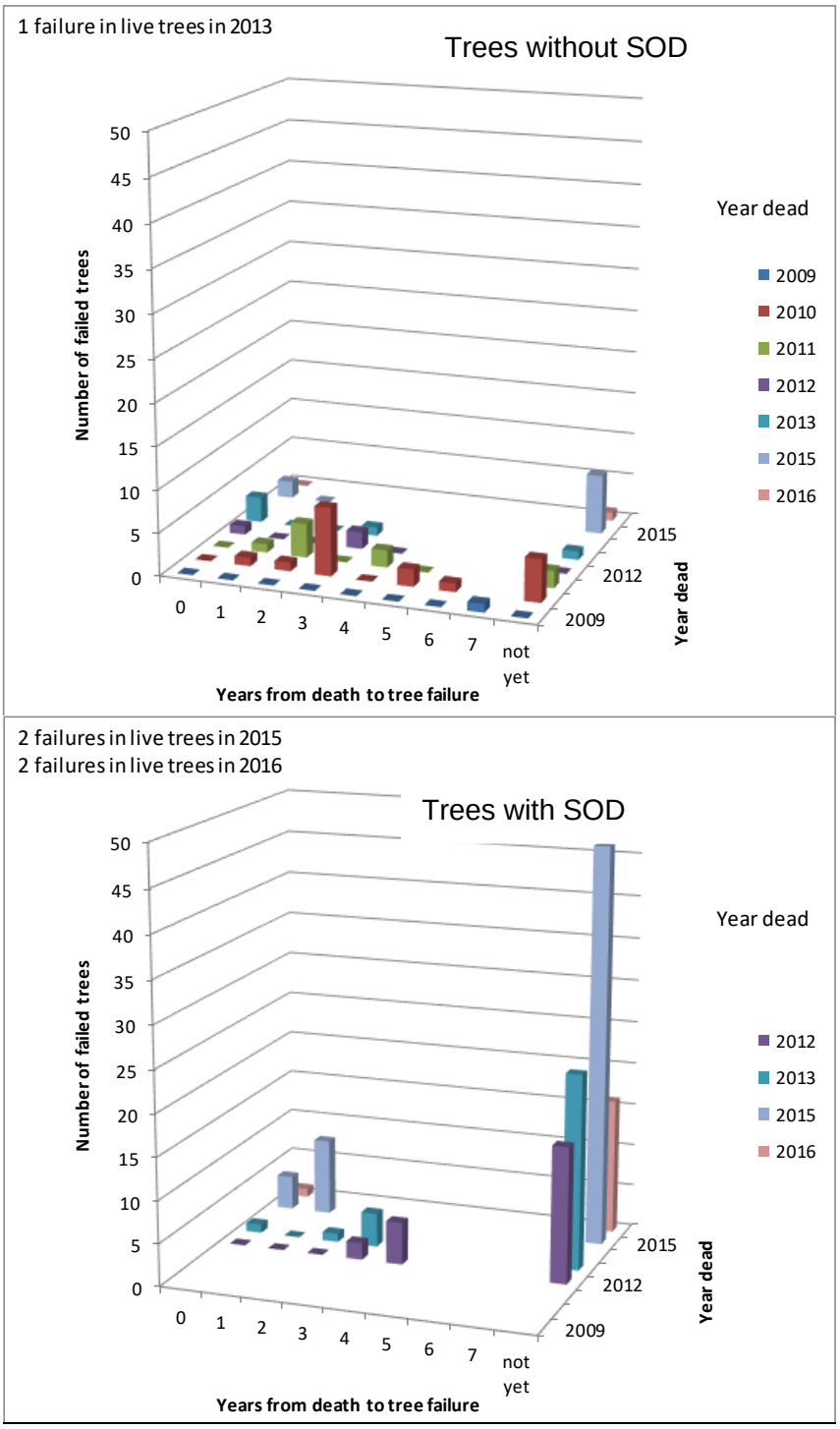

Figure 2-7. Comparison of years between tree death and failure for tanoaks with (bottom) and without (top) SOD.

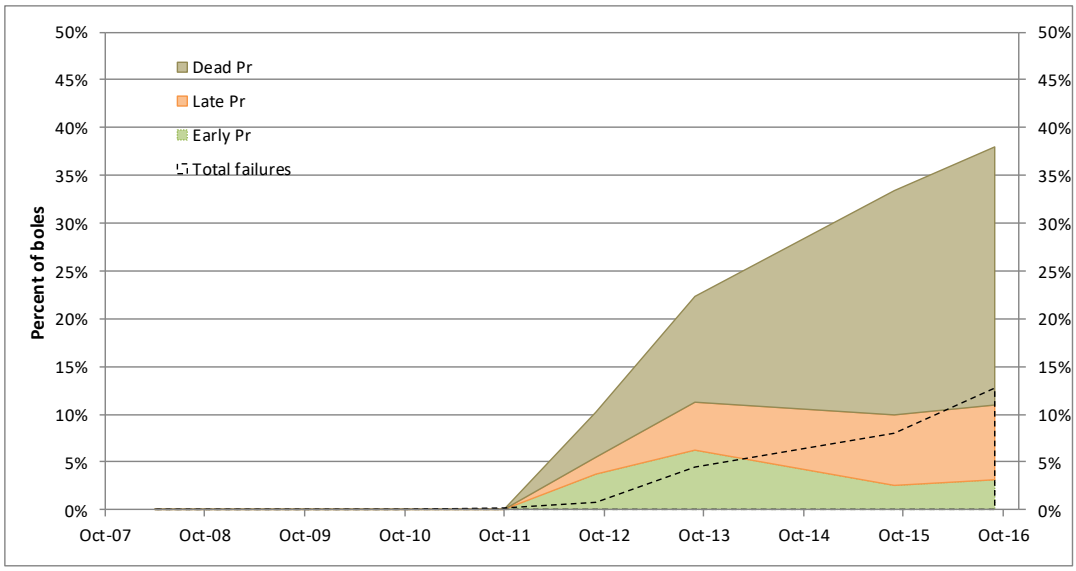

Figure 2-8. Progression of SOD symptoms and failures among monitored study trees over time. Dead Pr=dead due to SOD; Late Pr= late-stage SOD infection, beetles and/or *Annulohypoxylon thouarsianum*, Early Pr = early symptoms of infection only - bleeding bark cankers.

Figure 2-9. Branch structure and failure patterns among SOD killed tanoaks in the large tanoak trees in the Comstock Rd. phosphite trial.

3. Discussion

Reduced *P. ramorum* lesion expansion has been reported in phosphite-treated trees (Garbelotto and Schmidt 2009, Garbelotto et al. 2007). However, in this large trial, which used diameter-scaled dosages, phosphite stem spray application did not protect tanoaks from SOD. The initial invasion of the stand by *P. ramorum* was not impeded by the previous three years of phosphite stem-spray applications. In addition, no slowing of canker development or mortality was seen in phosphite-treated trees. In a previous study using similar study protocols on 63 treated and 133 control tanoaks in Sonoma County, we also observed no disease suppression from trunk spray applications of phosphite. In that study, controls also had lower levels of SOD incidence and mortality than tanoaks treated for 6 years with phosphite trunk sprays (Bernhardt and Swiecki 2013).

The observed lack of phosphite efficacy in tanoak treated by trunk spray application may be related to inadequate absorption of phosphite through the outer bark. The outer bark of mature tanoaks poses a significant barrier to phosphite absorption compared to the thin bark of young trees used in greenhouse tests. Even if some phosphite was absorbed by treated trees, it may not have accumulated to adequate levels in tissues where activity needs to be expressed, i.e., living phloem of the bole and in twig tissue. The distribution of SOD-affected trees within the treated stand and the lack of a preponderance of SOD-affected trees around plot edges in the treated population (Figures 2-4, 2-5, 2-6) suggest that phosphite application did not suppress foliar and twig infections or spore production in the canopy.

SOD incidence was significantly higher in the phosphite-treated trees than in the surrounding control areas (Figure 2-3). Differences in disease incidence across the study area appear to be related to the uneven spatial distribution of the disease across the stand (Figures 2-4 , 2-5, 2-6). The large differences in SOD incidence that can occur across a relatively small area, especially early in the local epidemic, could lead to misinterpretation of treatment effects on disease outcomes in studies using relatively small plots. One can reliably conclude that a treatment was not effective when high levels of disease develop among treated trees. However, the converse in not necessarily true in in studies that rely on natural spread of inoculum into plots. Without long-term monitoring, it is difficult to determine whether higher levels of disease in control plots compared to treated plots indicate a true treatment effect or are simply due to stochastic differences in initial inoculum distribution.

Infection of the tanoaks in this stand by *P. ramorum* has greatly increased the incidence of mortality and bole failure. A sizeable proportion of the SOD affected tanoaks in the stand that are dead have not yet failed (Figure 2-7, 2-8). Even among SOD-affected trees that have had bole failures, the failures are mostly so high in the trees that additional significant failures will still occur as decay weakens progressively larger diameter wood. As decay of SOD-killed dead trees proceeds, the number of root and root-crown failures will increase, although many trees are likely to be reduced to pole-like snags before they fail at the base. *Armillaria* and other wood decay fungi are likely to begin degrading the major roots and root crowns of these tree over time, which will increase the likelihood of lower-trunk failures.

Hazards associated with tree failure will persist for many years in this stand in the absence of active management to fell dead trees. To date, dead trees have mostly been removed if they could impact structures or fall across the access road / fire-break when they failed. In addition to this, SFPUC will need to fell additional dead trees to reduce hazards to those using or maintaining the trail that is being developed in this area. Although there has been a window of several years between SOD infection or mortality and elevated failure potential, it will be more difficult to track the failure potential of individual trees as additional infection events occur. Relatively prompt felling of recently killed trees should help reduce risks to tree crews that may be associated with unstable trunks that may break unexpectedly during felling operations.

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