Sudden Oak Death (SOD) Management and Monitoring in the Bay Area

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Cover photo: Tanoak mortality due to sudden oak death (SOD) in a phosphite-treated plot in Sonoma County.

Acknowledgements

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Summary

Part 1 of this report discusses tanoak plots set up to evaluate the effectiveness of phosphite trunk spray application for controlling sudden oak death (SOD). Tanoak plots were set up between 2005 and 2009 in Sonoma and San Mateo Counties and last evaluated in 2021. Drought conditions over multiple years of the study limited disease pressure overall, so SOD distribution was spatially patchy and generally low in many study plots. Because of this the distribution of disease in and around the plots needed to be evaluated to assess whether observed differences between treated and control plots were actually likely to be related to phosphite treatments. Overall, these studies did not provide evidence that phosphite applications had a strong effect on reducing *P. ramorum* infections in tanoak stands. When considered in combination with another large study that showed no efficacy of the phosphite trunk spray treatment for preventing SOD in tanoak, it does not appear that this treatment is a reliable or practical means for protecting tanoak stands from SOD.

Part 2 of this report discusses controlled studies funded under this project from 2008-2020, which showed that removal of California bay near susceptible oak species was highly effective in preventing new *P. ramorum* infections in susceptible oaks. Bay removal plots were located at Los Trancos, Monte Bello, Rancho San Antonio, and Russian Ridge Open Space Preserves and included SOD-susceptible stands of canyon live, coast live, and Shreve oaks. Over the study period, SOD incidence increased steadily among nontreated controls whereas only a few trees in areas treated by California bay removal became symptomatic. These results were previously presented in the June 2020 progress report and are included here for completeness.

Part 1. Effect of phosphite trunk spray application on SOD incidence in tanoak

1.1. Project objective

The objective for the USFS 2020-2021 contract year (1 July 2020 through 30 June 2021) was to evaluate phosphite treatments at 2 of 3 study locations listed below.

A. MROSD El Corte de Madera Open Space Preserve
B. Creighton Ridge, Sonoma County
C. Healdsburg, Sonoma County.

We initially selected locations A and B based on the fact that location C had not shown any new *Phytophthora ramorum* infections in recent years. In addition, all of the study plots in the Healdsburg location were burned in the August 2020 Walbridge (LNU Lightning Complex) fire.

1.2. Introduction

The objective of this study was to test methods for managing *Phytophthora ramorum* canker (sudden oak death [SOD]) in tanoak and oak stands. Because disease epidemiology differs between different canker hosts, we tested different control strategies in tanoaks and susceptible oaks. A comprehensive report covering the results of the oak portion of the study was previously submitted (Swiecki and Bernhardt 2020) and is repeated here as Part 2 of this report.

Landowners and managers are seeking ways to protect susceptible oaks and tanoak from SOD. Potassium phosphite (e.g., Reliant®, Agri-Fos®), a selective, systemic fungicide with a high level of environmental safety and very low non-target toxicity, has shown evidence of efficacy against *P. ramorum* canker in various greenhouse and limited field trials (Garbelotto et 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 et al. 2007, Garbelotto and Schmidt 2009). Because *P. ramorum* sporulates and can cycle on tanoak, fungicide application is one of the few strategies that has the potential to protect tanoak stands.

The exact mechanism by which phosphite controls *Phytophthora* is not fully 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 resistance reaction in response to infection (Guest and Grant 1991, Hardy et al. 2001). Phosphite application to tanoak could prevent disease by (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 contiguous area, because the proportion of treated trees surrounded by other treated trees will increase as plot size increases. Treatment of individual trees, or plots that are only a few tanoak canopies across, are unlikely to allow for optimum expression of reduced inoculum effects because inoculum from adjacent and nearby non-treated trees could be splashed and blown onto treated trees.

In this part of the project, we tested whether potassium phosphite, applied as a bark spray applied high on the bole, could protect tanoak stands from *P. ramorum* infections. Potassium phosphite has been widely used to protect susceptible trees from SOD, even though this use is supported by very little field data. The goal of our study was to test phosphite efficacy in a variety of sites under conditions that are realistic from an implementation standpoint. Phosphite applications were tightly calibrated and controlled in order to provide a reliable test of efficacy.

We first initiated field trials to determine how well phosphite works on tanoak under field conditions in 2005 with funding from the Kashia Band of Pomo Indians. Two trials were set up on private property within the ancestral range of the Kashia in northwestern Sonoma County in asymptomatic tanoak stands in SOD-affected areas. Additional field trials were set up in high-value stands on MROSD open space preserves and tanoak stands in Sonoma County (Table 1-1). The design of some of the later phosphite treatment plots was conducted collaboratively with Matteo Garbelotto (UCB) and Yana Valachovic (UCCE Humboldt Co) and was intended to allow for comparisons between plots established by the three research teams. To our knowledge, our plots were treated and monitored much longer than the others and no comparisons between the plots by the different research groups have been conducted to date.

In 2012, we determined that potassium phosphite trunk spray application failed to protect large-diameter treated tanoaks in a large-scale field trial on the San Francisco Peninsula (Swiecki and Bernhardt 2017). In addition, one of the phosphite trials we established in 2005 for the Kashia Band of Pomo Indians of the Stewarts Point Rancheria was discontinued in 2012 due to lack of efficacy (Table 1-1). That trial included both large and smaller diameter tanoaks. The field trials at El Corte de Madera OSP and the remaining plots in Sonoma County (Table 1-1) contain large numbers of smaller-diameter tanoaks. Our hope was that results from these projects could provide more definitive data on whether preventive phosphite trunk applications provide practical levels of protection against SOD in smaller-diameter tanoak.

SOD had been confirmed at low levels in tanoak plots at the Sonoma County locations when they were established, but SOD incidence remained stagnant due to prolonged drought conditions that persisted through the 2014-2015 rainfall year. Rainfall over the 2015-2016 winter-spring period was favorable for *P. ramorum* reproduction, and 2016-2017 rainfall was extremely high and conducive for *P. ramorum* reproduction and infection. Disease levels increased in the 2017 and 2018 evaluations at Creighton Ridge, but no significant treatment differences were apparent. We did not see disease increase at the location west of Healdsburg. At the MROSD tanoak phosphite study location, SOD was confirmed in two of five control plots for the first time since the study started in fall of 2017 and in the treated plot in February 2018. The 2017-2018 rainfall season was less favorable for *P. ramorum* reproduction, and only 1 new SOD infected tree was identified in fall 2018. Rainfall was moderate in 2018-2019, but drought conditions reappeared in 2019-2020 and became severe in the 2020-2021 rainfall year, which largely prevented new infections.

Table 1-1. Overview of tanoak phosphite-treated and control plots.

Study site	Locality	Plots	Phosphite applications	Notes	Last evaluation
CM	El Corte de Madera Open Space Preserve, San Mateo County	phosphite treated+thinned 158 trunks; nonthinned control 164 trunks	Jan 2009, May 2009, Nov 2009, Nov 2010, Nov 2011, Nov 2012, Nov 2013, Jan 2015,	One large (0.36 ha) treated plot with 4 smaller (0.11-0.17 ha) control plots around the periphery. Small understory tanoaks removed from treated plot only before start of study,	May 2021

Study site	Locality	Plots	Phosphite applications	Notes	Last evaluation
			Jan 2016, Dec 2016, Feb 2018, Feb 2019, Feb 2020		
SF	Seaview Ranch, Creighton Ridge area, Sonoma County	phosphite treated+thinned 63 trunks; thinned control 61 trunks nonthinned control 72 trunks	Dec 2005, May 2006, May 2007, May 2008, May 2009, May 2010, Oct 2011	Three matched plots (control, thin, thin+phosphite), about 0.05 ha each. Plot treatments ended in Oct 2011 due to the high amount of disease in the treated plot. Plots initially established under contract to Kashia Band of Pomo Indians.	September 2012. Observations terminated in 2012 due to high disease in phosphite treated plots
BL	Gualala Ranch Creighton Ridge area, Sonoma County	phosphite treated+thinned 57 trunks; thinned control 57 trunks; nonthinned control 56 trunks	Dec 2005, May 2006, May 2007, May 2008, May 2009, June 2010, Oct -Nov 2011, Jan 2013, Dec 2014, Jan 2016, Feb 2017, Feb 2018, Mar 2019, Feb 2020,	Three matched plots (control, thin, thin+phosphite), about 0.05 ha each.. Switched from spring application to fall/winter application. Plots initially established under contract to Kashia Band of Pomo Indians.	May 2021
PC	Gualala Ranch Creighton Ridge area, Sonoma County	phosphite treated + thinned control + thinned, 75 trees per plot. Landowner thinning designed to suppress fire risk during summer 2018 reduced each plot to 65 trunks.	Jan 2007, May 2007, May 2008, May 2009, May 2010, Nov 2011, Jan 2013, Dec 2014, Jan 2016, Feb 2017, Feb 2018, Mar 2019, Feb 2020	One treated (~0.09 ha) and control (~0.06 ha) plot pair. Understory tanoak mostly pre-thinned by landowner in both plots. Some minor additional thinning was conducted in treated and nontreated plots. Switched from spring application to fall/winter application. Plots established with funding from PSW-USFS as part of a collaborative project with M. Garbelotto and Y. Valachovic.	May 2021

Study site	Locality	Plots	Phosphite applications	Notes	Last evaluation
FE	Mill Creek Road, Healdsburg, Sonoma County	2 phosphite treated + thinned 36 and 34 trunks; 2 thinned control 30 and 41 trunks.	Feb 2007, May 2007, May 2008, May 2009, April 2010, Nov 2011, Jan 2013, Nov 2014, Jan 2015, Jan 2016, Jan 2017, Feb 2018, Mar 2019, Feb 2020	Two pairs of treated (~0.06 ha) and control plots (~0.08 ha)on one property. Understory tanoak mostly pre-thinned by landowner in all plots. Some minor additional thinning was conducted in treated and nontreated plots. Switched from spring application to fall/winter application. Plots established with funding from PSW-USFS as part of a collaborative project with M. Garbelotto and Y. Valachovic.	June 2020

1.3. Methods

Tree observations and data — At the start of the study, numbered aluminum tree tags were applied and DBH (diameter at 1.37 m above grade) and baseline tree health were recorded. Tree condition was assessed at the start of the study and annually thereafter in the late summer or fall. Tree diameters were remeasured in 2015 (BL, PC and FE locations), 2018 (El Corte de Madera), and 2021 (all locations except FE). 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 verified 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. Isolations were not conducted on material that was not suitable, such as cankers that were dried out. This was typically the case for trees that had died over the previous year. 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. 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. The presence of sporulation of *Diplodia corticola* was used to help differentiate cankers caused by this fungus from *P. ramorum* cankers.

Especially under low disease pressure, *P. ramorum* cankers do not commonly develop synchronously on all stems of multi-stemmed tree. Mortality due to SOD may occur on one or more stems while others of a multi-stemmed tree remain symptomless. Because individual main stems have different disease outcomes over time, we score and report SOD or other disease outcomes (e.g., *Diplodia*, *Armillaria*) on the basis of each main stem rather than the tree as a whole.

Phosphite treatments — Phosphite treatment was initiated with two applications spaced 6 months apart the first year and annual repeat applications afterward, as shown in Table 1-1. Fall applications were scheduled to occur after the first autumn rains. Phosphite was applied at the product label rate, i.e., a 22.36% a.i. aqueous solution (1:1 dilution of the concentrated product). Pentra-Bark® surfactant was added to the spray mix at the 2.3% v/v rate specified on the product label. Trunk diameters were used to calculate the amount of phosphite solution to apply to each trunk using methods described previously (Swiecki and Bernhardt 2007, 2013). 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. After tree diameters were remeasured in 2015 and 2018, application volumes were adjusted as needed based on the new diameter measurements.

To maximize the opportunity for phosphite uptake, we banded the spray high on the stem (3 to 6 m height). This high bark application provides two main advantages that should increase phosphite uptake:

1. Material is applied where the bark is thinner (and presumably more permeable) and generally less densely coated with mosses that may absorb and tie up the phosphite.

2. As the residue is remobilized by rain and moves downward, it has additional opportunities to be absorbed in the lower portion of the stem before being washed into the soil.

Phosphite at El Corte de Madera OSP was applied by Mayne Tree Expert Company (San Carlos, CA) and in later years by West Coast Arborists under contract to MROSD (Figure 1-1). Ted Swiecki provided quality control for these applications and calculated the actual amount of spray applied. Applications were made using a 95 L spray tank mounted on an ATV with a 12 VDC electric pump. Phosphite applications at the SF, BL, and PC locations for the first several years were treated using the Kashia Band's ATV and spray tank. Later applications were made using a 12 L spray tank with a 12 VDC electric pump mounted on a modified mountain bicycle (Figure 1-2, 1-3) The FE location is too steep for an ATV and from the beginning was treated using the bicycle-mounted spray apparatus.

In all instances, 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. The spray was banded on the trunk starting at a height of about 6 m and applied downward, typically creating a band extending at least 3 m down the trunk. 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 measured by comparing 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 at El Corte OSP took 2 to 3 days by the 3-person crew and were completed within a one week period. Applications at the FE site in Sonoma County were conducted in one day with a two-person crew. The BL, PC, and SF locations are relatively close together, and could be treated over the course of 2 days by two people using the bicycle-mounted sprayer. After treatment was discontinued at SF, BL and PC were treated on the same day.

Tree removal to increase defensible space near the residence at the PC location was conducted sometime between our June 2018 and March 2019 site visits. The plan for this work was to remove small diameter tanoaks (up to about 23 cm DBH) within a set radius of the residence. The tree removal area included portions of both the control and treated plots. The treated plot was closest to the residence so that if the phosphite treatment was effective, the landowners would achieve maximum benefit.

Tree removal work had much less impact on the study plots than originally feared. Extensive clearing near the residence effectively moved the stand edge closer to one edge of the phosphite-treated plot than it was initially. Fuel reduction tree removal did not substantially change the position of the stand edge relative to the control plot.

Ten of the original 75 trunks in the PC control and treated plots were felled. Similar sized trees were removed in each plot. Nine felled trees in the control plot were less than 23 cm DBH and one was 33 cm DBH. In the treated plot, nine felled trees were less than 15 cm DBH, and one tree was 35.5 cm DBH. None of the felled trees were dead; 2 trees with SOD were felled in the control plot, 1 tree with SOD was felled in the treated plot. These trees have been included in the counts of symptomatic trees.


Figure 1-1. West Coast Arborists applying phosphite at El Corte de Madera using ATV-mounted sprayer.

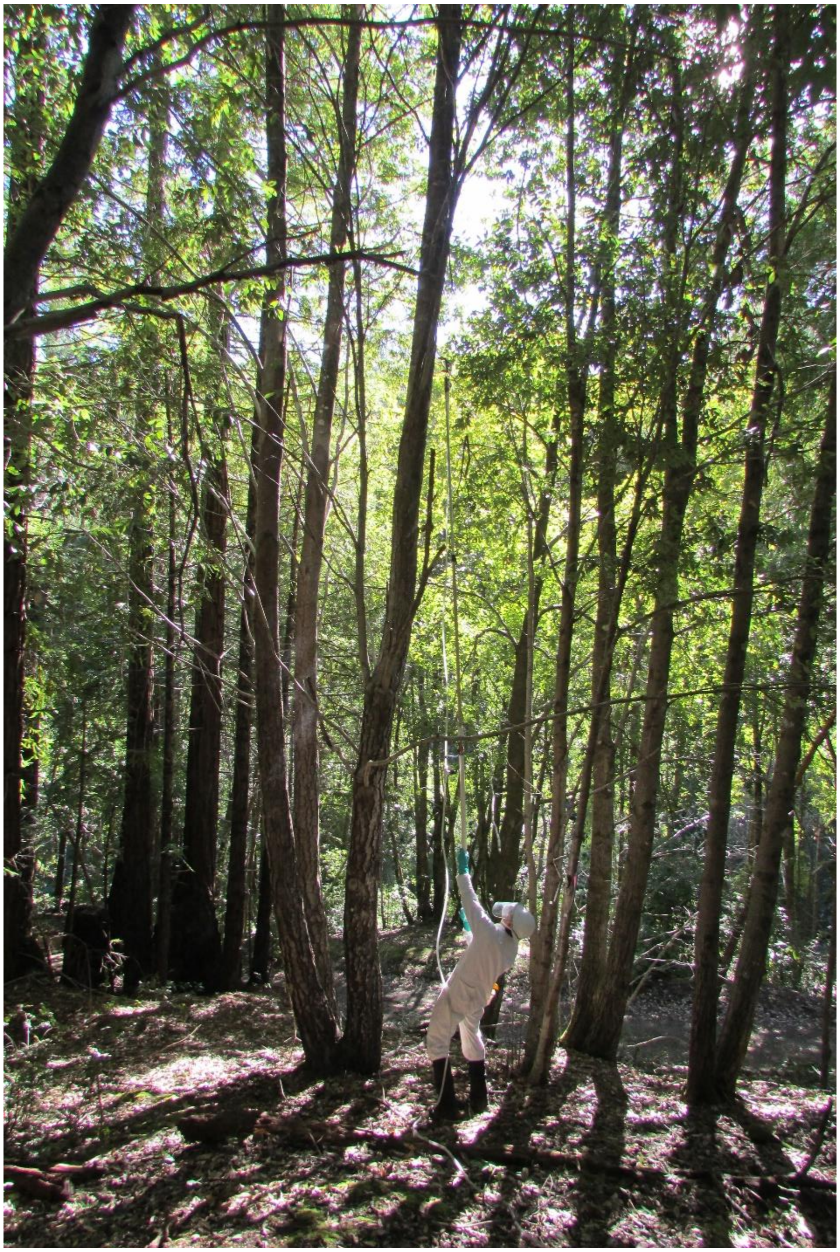


Figure 1-2. Ted Swiecki applying phosphite to tanoaks at the lower FE plot using the bicycle-mounted sprayer.

Figure 1-3. The bicycle-mounted sprayer uses a 12 VDC pump/speed controller/battery assembly mounted on the back rack (under white housing) that is activated by an electric switch (gray electric box at end of black cord by top of tank). The telescoping spray pole, hoses and other components are attached to racks and other points on the bicycle for transport. A rope was attached at the front or back to allow a second person to belay or assist in pulling the bike on steep terrain.

1.4. Results

1.4.1. El Corte de Madera Open Space Preserve

At this location, we compared SOD levels in a large (0.36 ha) contiguous block of trees treated by bark application of phosphite with untreated trees in 4 adjacent or nearby blocks (Figure 1-4). A single large treated block was used instead of multiple smaller treated blocks because larger blocks should maximize any effect of phosphite on reducing local inoculum by reducing foliar and twig infections. The control plots were distributed around the treated block to the degree possible so that spatial gradients in disease pressure could identified more readily.

All blocks were dominated by tanoak overstory with a few Douglas-fir trees, but some highly suppressed *Quercus parvula* var. *shreveii* and *Q. chrysolepis* were located in portions of the treated plots and some of the control plots. These SOD-susceptible oaks were also treated within the treated plot. The initial mean trunk diameter of plot trees was 26 cm. Most of the tanoaks in the control and treated plots had a single main stem, but multi-stemmed tanoaks were more common in the treated plot (24%, 25/104) than in the control plots (13%, 17/127) at the start of the study.

The total target volume of spray mix decreased over the years due to death of some trunks in the plot. The last phosphite treatment was applied 14 and 17 February 2020 (Table 1-1). In all, we applied 136 L of spray mix, made with 66.6 L of Reliant phosphite fungicide and 3.2 L of Pentra-Bark surfactant. Reliant is 0.62 kg potassium phosphite per L (45.8% active ingredient), so the total application was 41.2 kg of potassium phosphite.

Final disease evaluations were made on 12 May 2021. When these plots were established in 2008, they were thought to be at high risk of developing SOD within the next several years. However, spread of SOD into the plots was much slower than anticipated, presumably due to drought conditions which prevailed for many years after plot establishment. At the September 2016 plot evaluations, SOD infections were confirmed in tanoaks and California bay located to the northwest, about 120 m and 160 m from the edges of the nearest control plot and the treated plot, respectively. Tree mortality in the plots through September 2016 was observed primarily in somewhat suppressed understory trees and was mostly associated with *Diplodia corticola* trunk cankers, although *Armillaria* cankers were also observed on several tanoaks.

After the historically rainy winter of 2016-2017, SOD symptoms were seen in the plots for the first time during the disease evaluations which were conducted in October 2017 (Figure 1-5). SOD symptoms have increased in a discontinuous and patchy fashion across the area in the succeeding years resulting in only scattered patches of SOD-affected trees in the plots.

At the May 2021 evaluation, SOD symptoms were still relatively uncommon and spatially clustered in the study area (Figure 1-4). Two of the four control plots each had 5 SOD-affected tanoak trunks; one control plot had 11 SOD-affected tanoak trunks; and no SOD was found in the remaining control plot. *P. ramorum* was isolated from 8 of the 21 symptomatic trunks. Only one tanoak in the phosphite-treated plot developed clear SOD symptoms by May 2021. *P. ramorum* was isolated from this symptomatic trunk. Considering all tanoak trunks in the treated and control plots, SOD incidence though 2021 was significantly higher overall ($p<0.0001$, Fisher-Boschloo exact test) in the controls (15%, 21/138 trunks, exact binomial 95% confidence interval 9.67-22.32%) than in the phosphite treated plot 0.75%, 1/134 trunks (exact 95% binomial confidence interval 0.02-4.09%). Percentages are based on the number of trunks that were live in 2017, the first year that SOD symptoms were observed within the plots.

Even though the overall difference is statistically significant, our overall confidence that this difference represents a treatment effect is low because of the low numbers of symptomatic trunks and the obvious spatial clustering of SOD in the plots. Control plot 3, which was at the edge of a large SOD hotspot that developed within the past few years, had a significantly higher incidence of SOD in tanoak (11/25 = 44%) than any of the other controls (Figure 1-4, 1-5). In phosphite-treated plots at two other locations where no efficacy was seen (SF from this study and the large SFPUC tanoak phosphite study, Swiecki and Bernhardt 2017), SOD incidence in the phosphite-treated plots was significantly greater in the control plots initially and continued to be so for multiple years, even though there is no evidence that phosphite treatment increases the likelihood of *P. ramorum* infection. Given the scattered spatial distribution of SOD in this location and the overall low SOD incidence in the study area, it is possible, if not likely, that differences in incidence between treated and control plots at El Corte through 2021 were due to chance.

SOD symptoms were seen across a range of tanoak stem diameters (Figure 1-6). Large diameter stems had higher symptom incidence than small stems. In a logistic regression of the 2021 binary SOD outcome, both treatment and diameter were significant (likelihood ratio p<0.0001 and 0.0353, respectively). No SOD symptoms have been observed among the small number of suppressed Shreve and canyon live oak trees within the control (7 trunks) and phosphite-treated (11 trunks) plots.

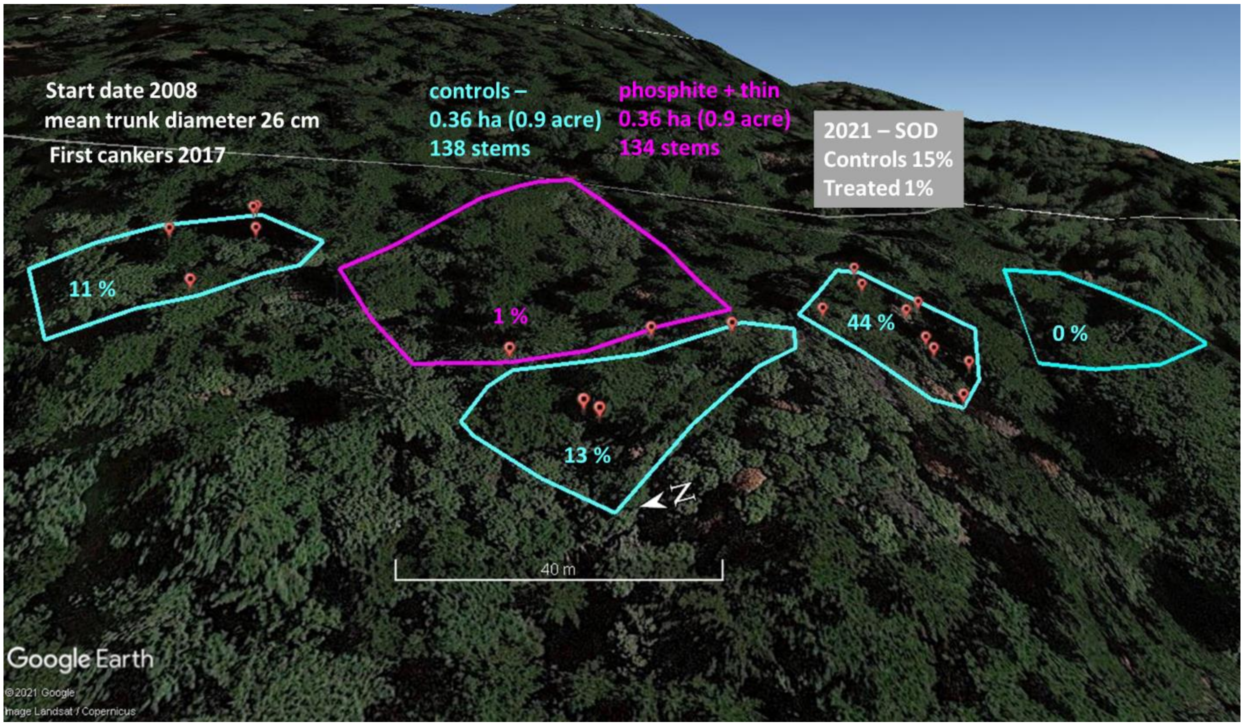


Figure 1-4. Locations of tanoaks with SOD canker symptoms (red pointer icons) and SOD incidence by plot (percentages) as of May 2021 at El Corte de Madera Open Space Preserve. Controls are monitored in four plots, numbered 1 to 4 from left to right. Cyan = control plots, fuchsia = treated plot. Percent SOD incidence in plots is based on the number of live tanoak trunks in 2017 (shown for each treatment), when SOD cankers were first observed in the plots. Note dead tanoak canopies downslope and west of controls 2 and 3. Imagery date 9/6/2020.

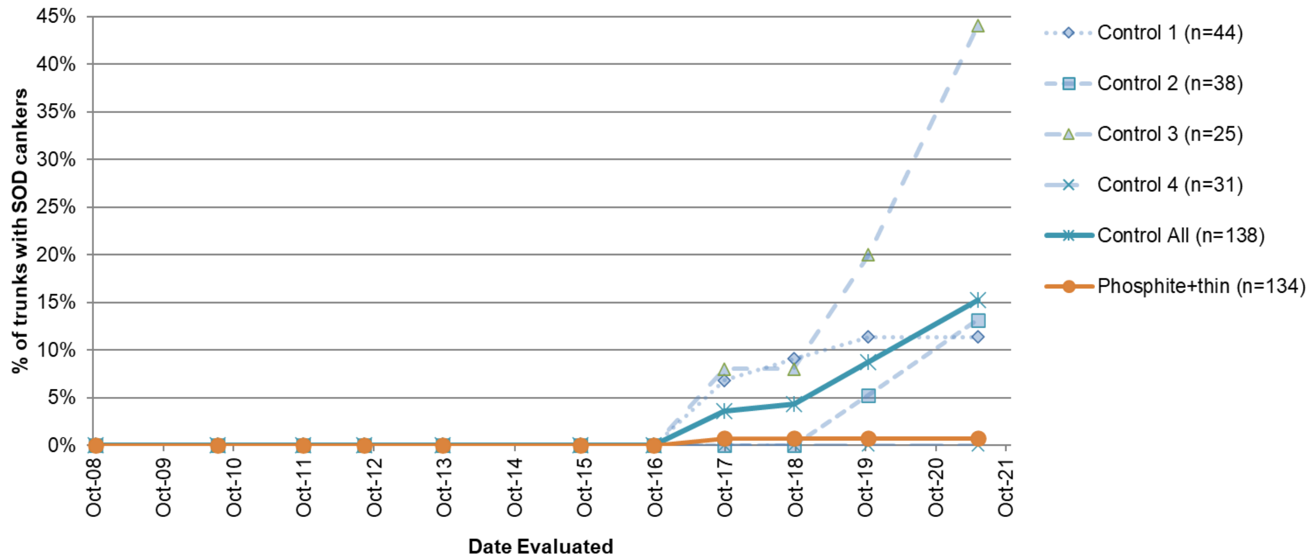


Figure 1-5. Percent of tanoak trunks with *Phytophthora ramorum* cankers in El Corte de Madera Open Space Preserve control and phosphite-treated plots. Number of live trunks per plot as of 2017 (first year of SOD in plots) was used to calculate percentages.

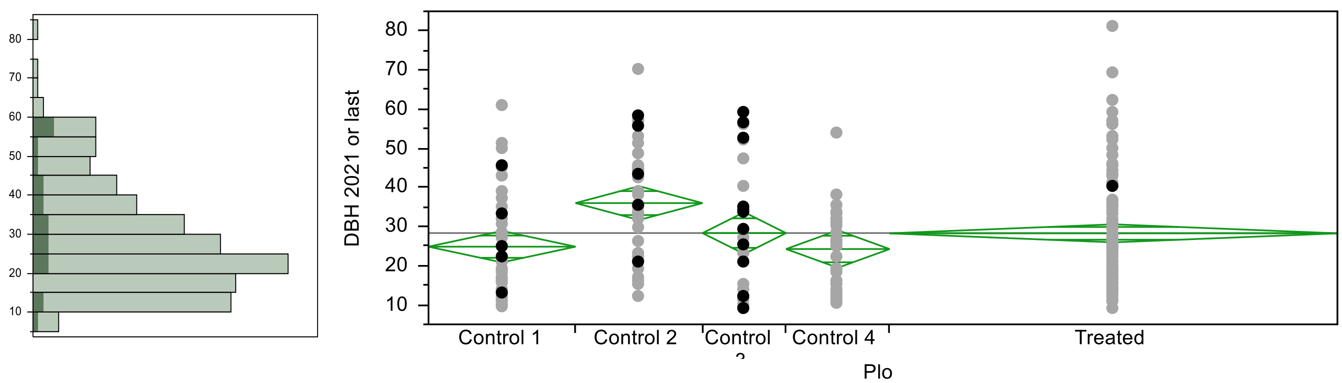


Figure 1-6. Diameter and SOD status in 2021 of tanoak stems in the control and treated plots. Left: overall distribution of stem diameters with shaded portions indicating stems with SOD symptoms. Right: Means and distribution of stem diameters in each plot. Stems with SOD symptoms are shown as black points. Mean stem diameter is center line of green diamond, upper and lower points indicate the 95% confidence interval, width of diamond is proportional to the number of stems. Horizontal black line is the overall mean.

Tanoak mortality. Tanoak mortality was observed in all plots within the first few years of the study, up to 8 years before the first SOD infections were documented (Figure 1-7). Over the entire study period, most tanoak mortality has been due to causes other than SOD, including tree failures, Armillaria root disease, and trunk cankers caused by *Diplodia corticola* and possibly other fungi, especially among smaller-diameter trees (<20 cm DBH). Levels of non-SOD tree mortality in treated (12%) and control (13.4%) plots were similar. Of the 38 tanoak trunks that died in control plots since the study began, 16 died due to SOD. This is 42% of all tanoak mortality in the control plots (42%, 44%, 50% and 0% of mortality in control plots 1 through 4, respectively). In the treated plot, 20 trunks have died, one due to SOD (5% of all mortality). Although none of the highly suppressed oaks in the plots developed SOD symptoms, 40% (8/20) of the Shreve oak and 57% (4/7) of the canyon live oak stems died over the study, mostly as the result of severe canker rot.

Death of SOD-affected tanoaks was first seen in 2018 evaluations, 1 year after *P. ramorum* cankers appeared in the plots. Many of the SOD-affected tanoaks died rapidly after infection. Of the 21 control trunks with SOD, 16 (76%) died by May 2021. The SOD-affected tanoak trunk in the treated plot also died; phosphite treatment did not prolong its survival relative to similar infected trees in control plots.

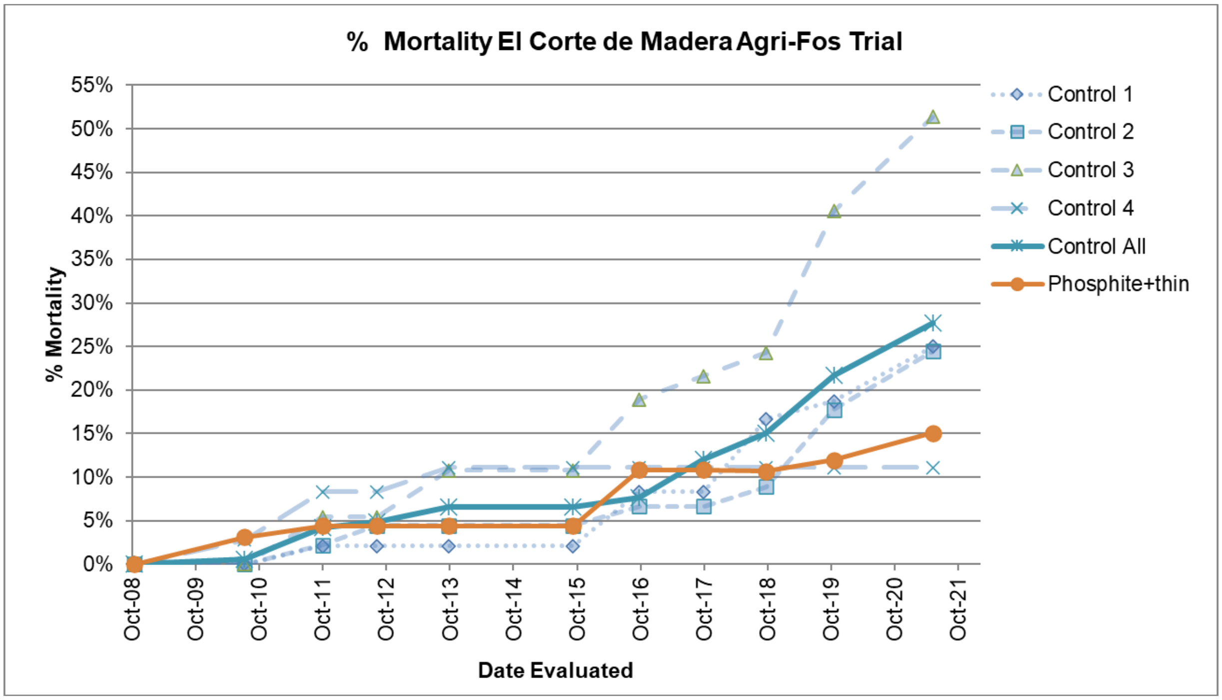


Figure 1-7. Mortality over time (% of tanoak trunks) at El Corte de Madera Open Space Preserve based on number live trunks at the start of the study in 2008 (157 trunks in phosphite treated plot, 166 trunks in control plots). Counts exclude two trunks removed from the treated plot within the first year due to damage from an adjacent root failure. Most mortality is due to Armillaria root disease or *Diplodia corticola* cankers. Death due to SOD was first seen in October 2018, the year after *P. ramorum* cankers were first seen in the plots.

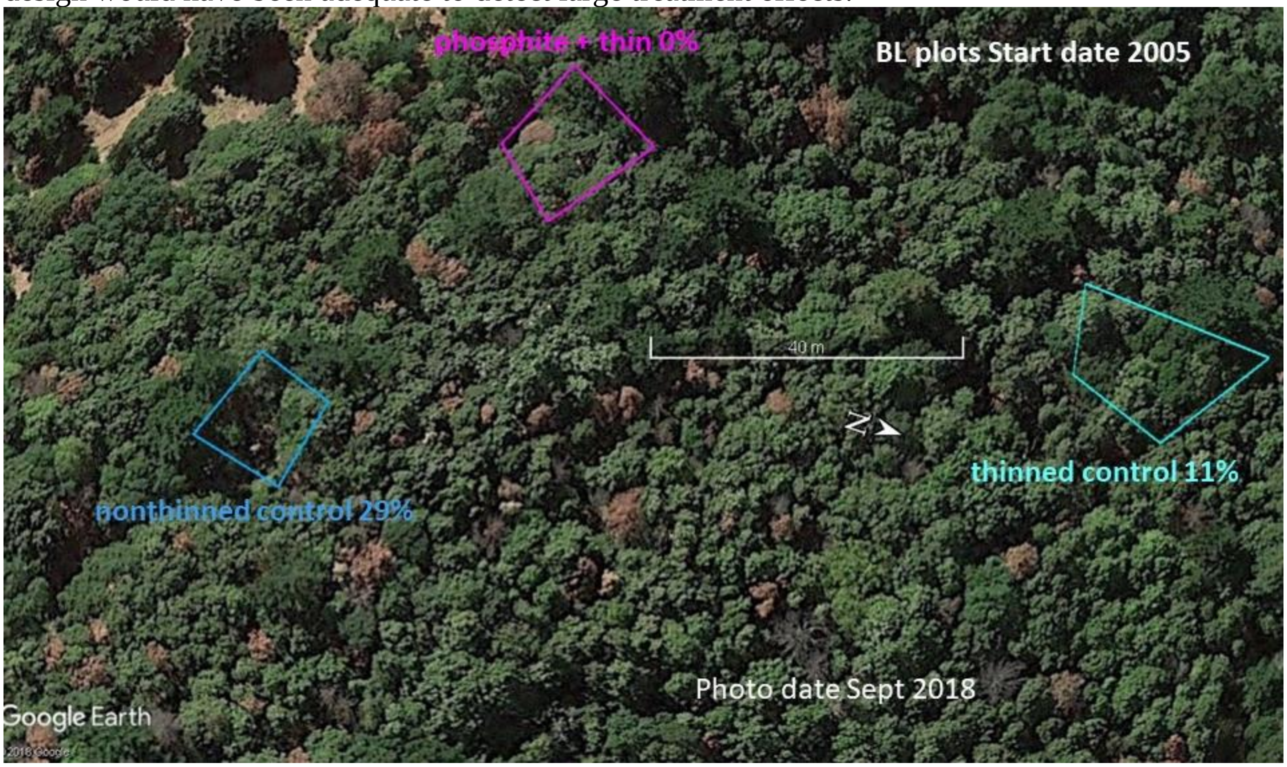
1.4.2. Sonoma County plots

At the Sonoma County sites (Figure 1-8). treated trees were mostly small-diameter tanoaks in relatively small plots. Two sets of plots are in the Creighton Ridge area in northwestern Sonoma County about 0.55 km apart, located on separately owned parcels (BL, PC). The other two sets of plots are on a single parcel (FE) west of Healdsburg, about 20 km to the southeast. Two pairs of treated and control plots about 0.21 km apart were established at the FE site. Study plots in Sonoma County were last treated with phosphite on 24 and 28 February 2020.

Figure 1-8 Sonoma County plot locations.

<u>Northwestern Sonoma County plots: BL, PC, and SF</u>

Tanoaks with *P. ramorum* cankers were present within 100 m of plot sets when they were first established. Disease in Sonoma County increased noticeably due to the record rainfall in 2016-17, but was still distributed in a patchy manner across the landscape in plot locations. This is evident from aerial images (Figure 1-9). Because of this patchy spatial distribution, the percent SOD infection in any given untreated plot is highly dependent on its location. SOD incidence in the control plots would have been substantially higher or lower disease percentages if plot locations were shifted by as little as 10 m in various directions. In the plot design used at El Corte de Madera (discussed above) the use of multiple large control plots allowed us to document the spatial variation in disease incidence throughout the study area. Although the Sonoma plots were set up in a paired fashion and matched to similarities in stand composition to the extent possible, this design does not adequately control for the spatially stochastic nature of disease development in areas where *P. ramorum* had not yet become established at the start of the study. However, at the time these plots were established, it was anticipated that high disease levels would develop throughout these nearly pure tanoak stands. If that had occurred, the plot design would have been adequate to detect large treatment effects.



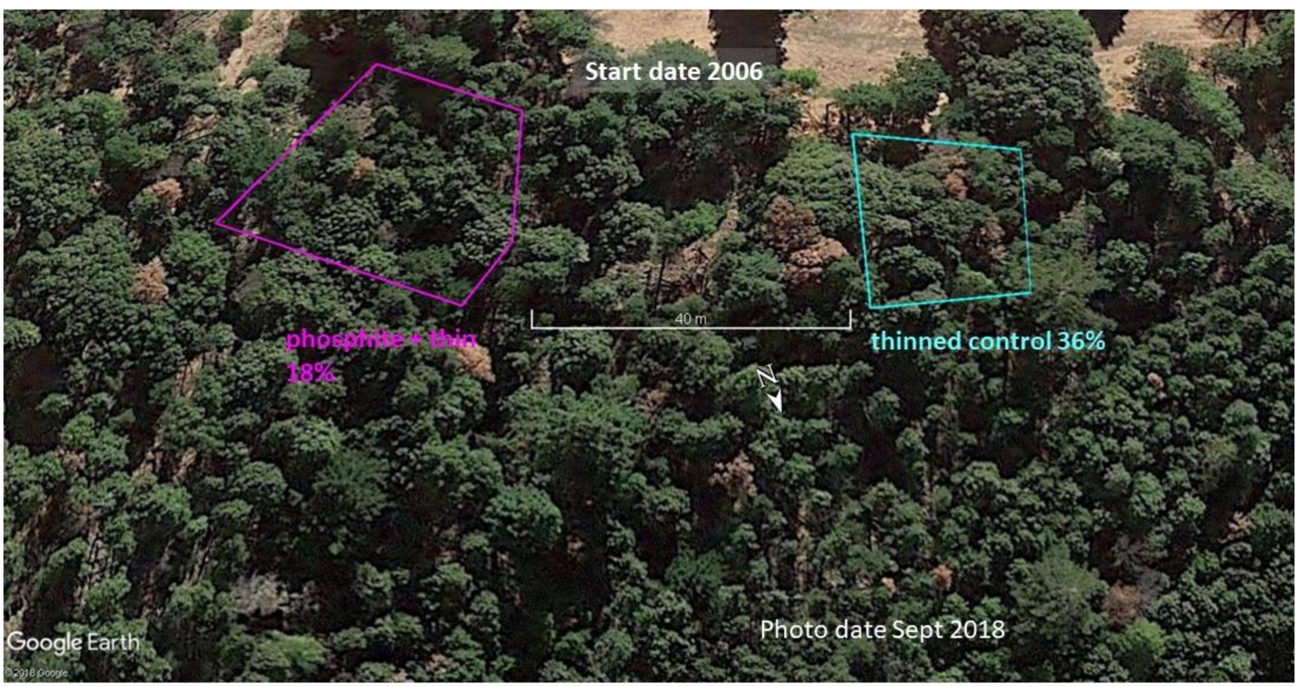


Figure 1-9. Patchy distribution of SOD in study locations BL (top) and PC (bottom). Percent SOD infection of tanoaks in phosphite-treated and control plots is as of May 2021.

Final disease evaluations were made at the PC and BL plots in May 2021. Disease progress over time is shown in Figure 1-10. SOD incidence increased most dramatically in the nonthinned control plot at BL in the two years after the wet 2016-2017 rainy season. SOD incidence in the other two plots (thinned control and thinned phosphite-treated) did not show a strong increase in disease following the wet 2016-17 rainy season (Figure 1-10), and do not differ significantly from each other. Both winter 2019-2020 and 2020-21 were relatively dry, and no additional infected trees appeared in any of the BL plots after 2018. Bleeding cankers from which *P. ramorum* was isolated that subsequently became inactive and callused over account for small reductions in disease symptom incidence over time in Figure 1-10.

From field observations and aerial images (Figure 1-9, note dead tanoak canopies), it was evident that disease incidence was variable across the study plot area and that the nonthinned plot was in a localized patch with high SOD incidence. Although the difference in disease incidence between the nonthinned plot and the other two plots could be a treatment effect, we think it is more likely that the differences are due to chance, given the spatial variability in disease at this location.

The plots at PC are slightly closer to the ocean than the BL plots and are just over the top of a ridge. At PC, both treated and control plots showed an increase in SOD incidence in response to the 2016-17 rains, and new disease symptoms continued to appear through 2020 (Figure 1-10). Data analysis is complicated by the fact that some trees were removed from both plots in later 2018 (see methods), effectively censoring some of the observations. SOD incidence in 2021 was higher in the thinned control plot than in the phosphite-treated plot, but the binomial confidence limits were overlapping.

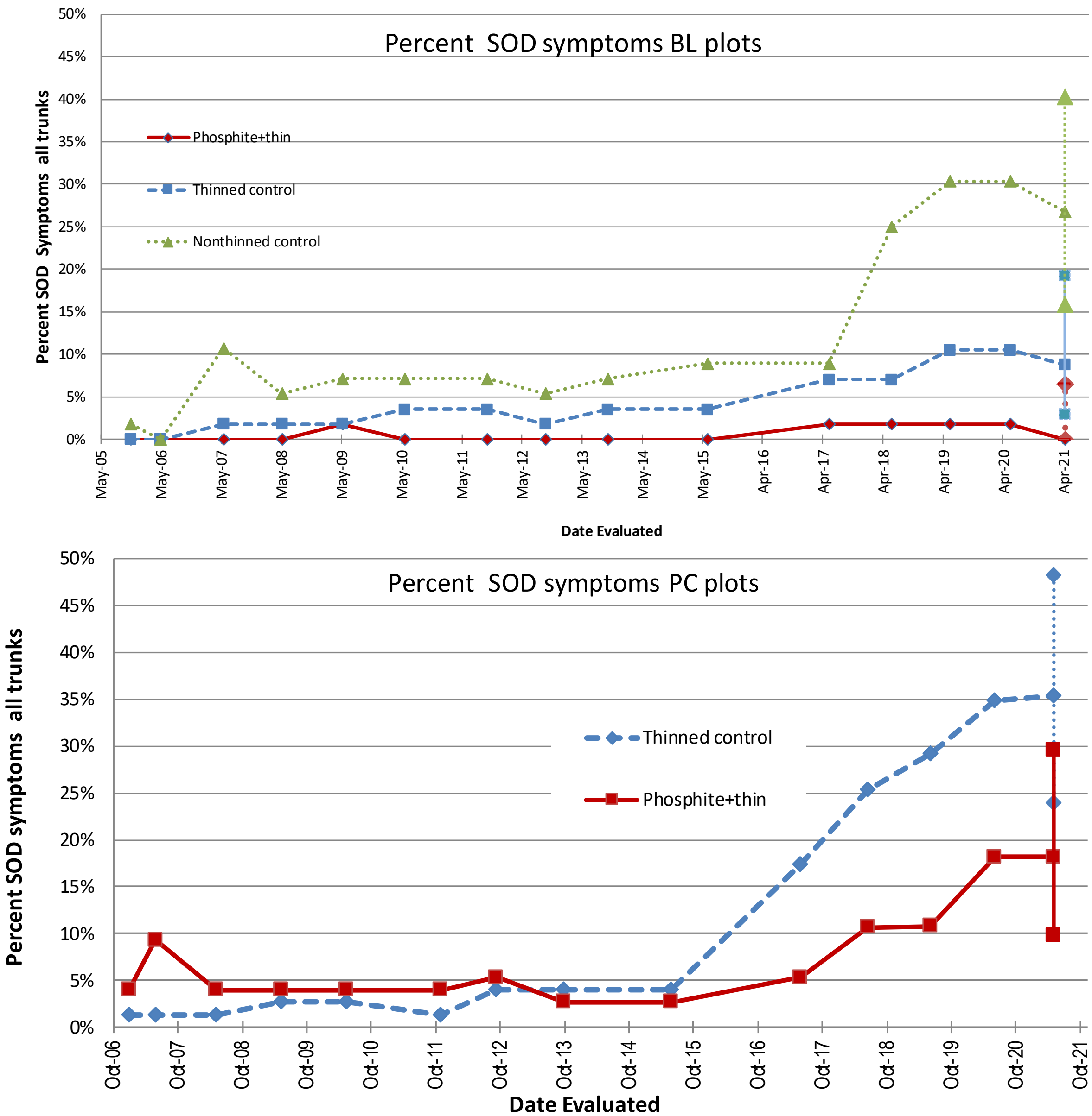


Figure 1-10. Percent of tanoak trunks diagnosed with SOD over time at BL and PC plots. N=56-57 trunks per plot at BL, N=75 for both treatments at PC until 2019 when N=66 for both treatments due to tree removal. Vertical lines represent exact binomial 95% confidence limits for 2021 data.

Mortality. Mortality has increased steadily in the BL control plots (Figure 1-11), due primarily to *Diplodia*-type cankers on small suppressed trees. As shown by the binomial confidence limits, there is significantly less tree mortality in the treated than in control plots. None of the 4 tanoak stem deaths in the phosphite-treated plot was attributed to SOD. This plot also had 3 tanoak trunks that had been cut down by the landowner due to partial failures and were excluded from analyses. Three of 18 dead trees in the thinned control and 14 of 17 dead trees in the nonthinned control were attributed to SOD.

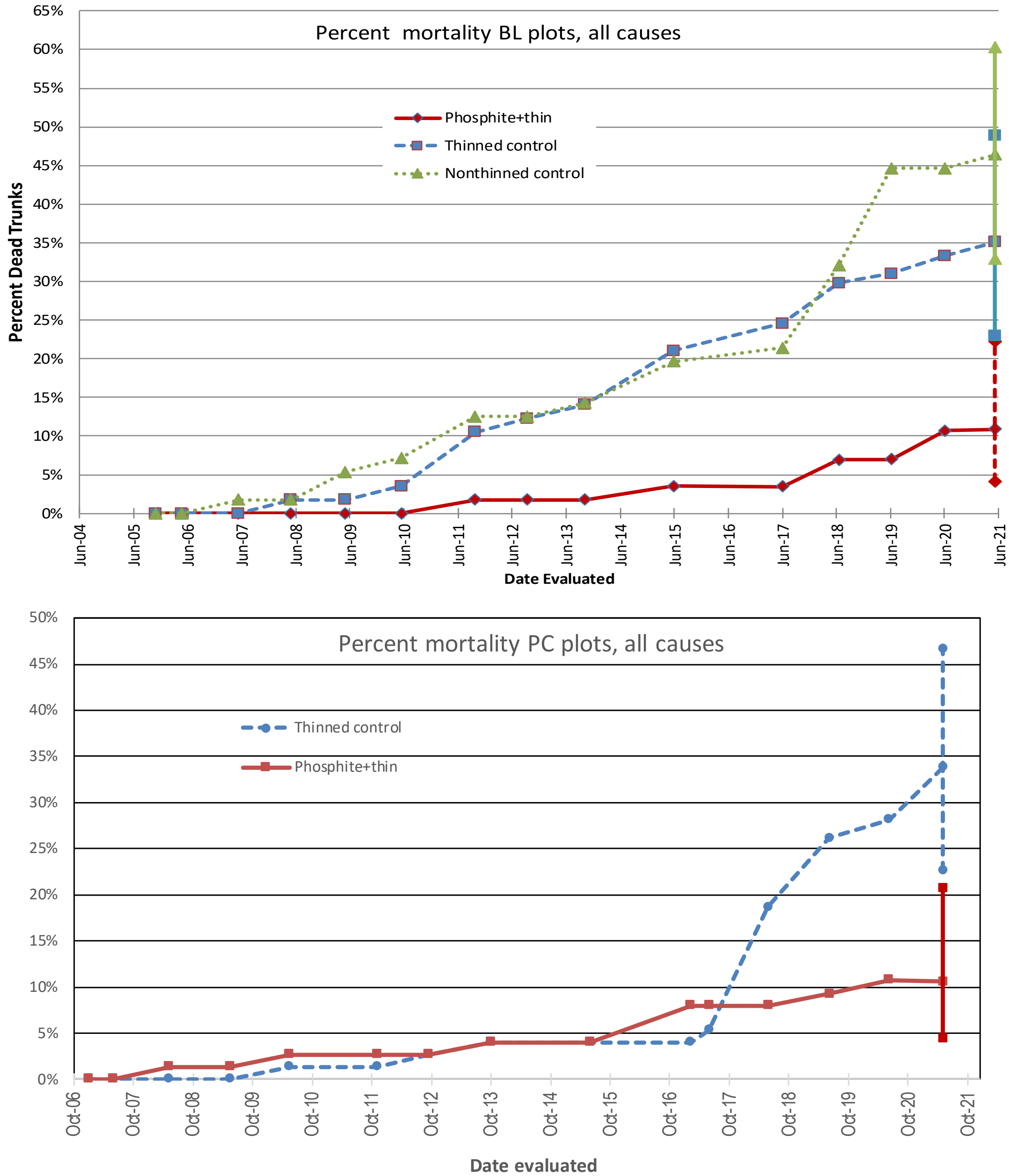
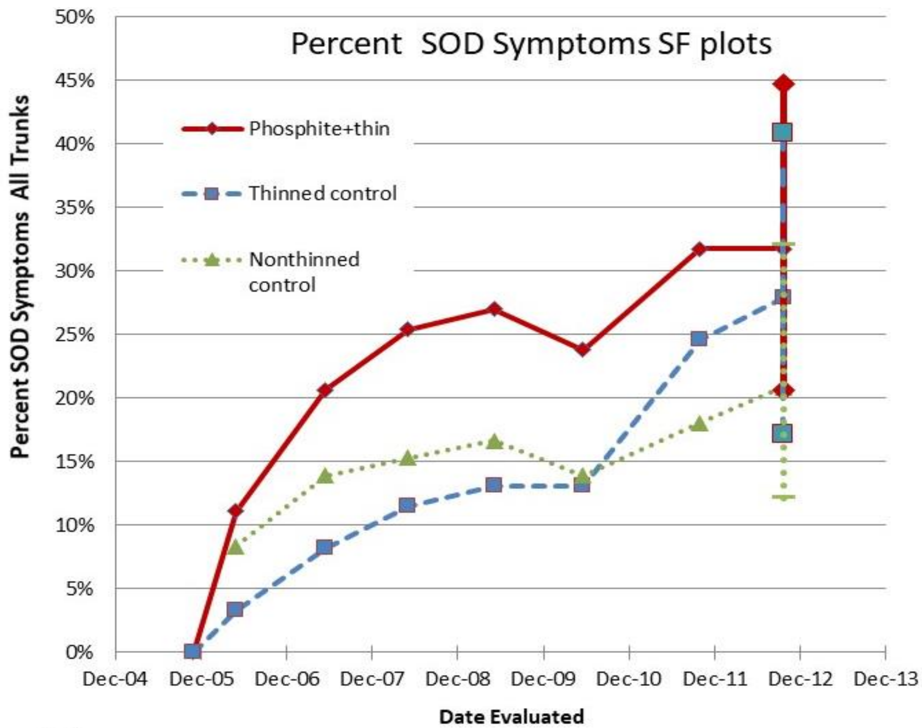


Figure 1-11. Percent of dead tanoak trunks (bottom) over time at BL and PC plots. N=55-57 trunks per plot at BL, N=75 (both treatments) before tree cutting and 65-66 afterward at PC. Vertical lines represent exact binomial 95% confidence limits.

Due to a much lower incidence of *Diplodia*-type cankers, overall mortality at the PC plots was lower than at BL (Figure 1-11). As shown by the binomial confidence limits, there is significantly less tree mortality in the treated than in control plots. SOD is the main factor associated with tanoak mortality at this location. Four of six dead (67%) trees in the phosphite-treated plot and 14 of 17 (82%) in the thinned control plot were due to SOD.

SF plots. The SF plots in the Creighton Ridge area had the highest amount of SOD in the plot vicinity at the time that the study was initiated. The last phosphite treatment was in fall 2011 and data collection was discontinued after 2012 because high levels of SOD and SOD mortality had developed in the phosphite-treated plots (Figure 1-12). SOD incidence seen in the phosphite + thin SF plot in 2012 (Figure 1-12) was similar to that observed in the most affected plots at PC and BL in 2021 (Figure 1-10). Although all plot trees were asymptomatic when plots were established, SOD symptoms began to appear within the first 6 months of the study (Figure 1-12), indicating that some of the plot trees could have been infected the previous season or earlier. However, all plots showed a second pulse of symptom development after 2009 associated with a later infection period five years after the start of the study (Figure 1-12).



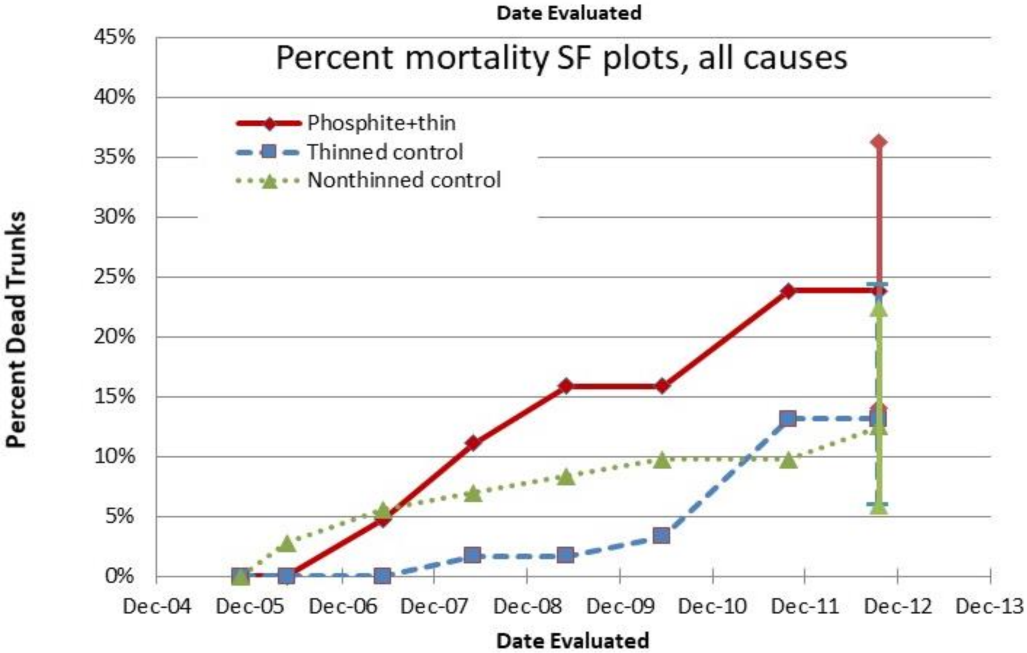


Figure 1-12. SOD disease progress and all mortality in tanoaks at SF plots through 2012, when plots were discontinued. Error bars are exact binomial 95% confidence limits.

Aerial images (Figure 1-13) show that tanoak mortality due to SOD developed more rapidly and extensively at this location than at the nearby BL and PC plot locations (Figure 1-11). The SF, BL and PC locations are similar distances from the coast, but it appears that California bay is more abundant in the vicinity of the SF compared to the BL and SF plot locations. Inoculum produced on California bay increases SOD incidence and severity in tanoak, which may result in more uniform disease pressure in locations such as SF. Even with the greater disease pressure at SF, examination of historical aerial imagery in the area shows that mortality has been somewhat patchy over small and larger spatial scales.

Figure 1-13. (next page) SF plot outlines superimposed on aerial images from June 2005 (top, before start of study), July 2012 (center, last year of data collection), and Sept 2018, showing progress of SOD mortality in and around plots.


2005
N
SF1
SF2
SF6
90 m
2012
N
SF1
SF2
SF6
90 m
2018
N
SF1
SF2
SF6
90 m

Tree diameter and SOD

At the PC and SF locations, the presence of SOD symptoms was positively correlated with DBH (Figure 1-14). Larger diameter stems were more likely to have developed SOD (logistic regression likelihood ratio p=0.0004 and p<0.0001, for PC and SF respectively). However, *P. ramorum* infections were seen even in the smallest size class stems in the treated plots at PC and SF. This suggests that phosphite did not prevent infection even in small stems, which should have absorbed the largest relative phosphite doses. The relationship between DBH and SOD was not significant at the BL location, likely due to the low overall SOD incidence at this site and small number of stems more than 40 cm DBH.

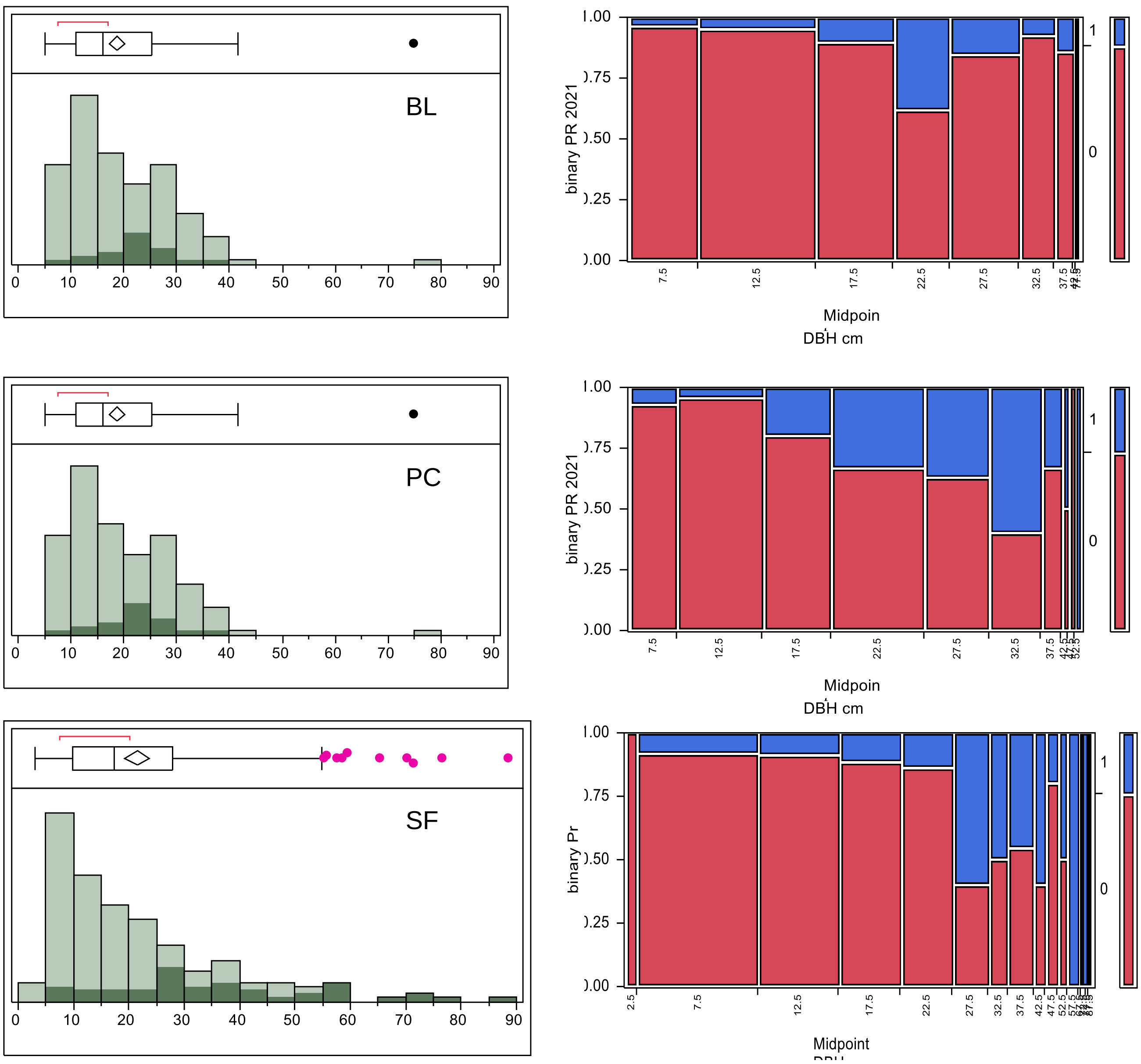


Figure 1-14. Diameter and SOD status of tanoak stems in 2021 at BL (top) and PC (center), and in 2012 at SF (bottom) plot locations. Shaded portions of bars indicate stems with SOD symptoms.

Healdsburg plots: FE

The FE plots are west of Healdsburg in a hotter, drier, more inland location compared to Creighton Ridge (Figure 1-8). Plots had a wide distribution of stem diameters, ranging from about 5 to 48 cm DBH, but most stems were 20 cm DBH or less. Mean stem diameters in the two lower plots (21 and 20 cm for treated and control, respectively) were slightly larger than in the two upper plots (18 cm for both). The two pairs of treated and control plots at this location are about 0.2 km apart. SOD-infected tanoaks were observed 100 m from these plots when they were first established. SOD has remained at low levels in and near the plots, as can be seen from the predominantly green canopies in the September 2018 aerial photo (Figure 1-15). Very little disease was been detected in the plots over the 13.5 years of monitoring (Figure 1-16). Furthermore, no new disease was seen between 2013 and 2020, a time period in which disease increased in at least some plots at the other tanoak study locations. No significant differences in SOD incidence were seen between plot locations (upper vs. lower) or treatments.

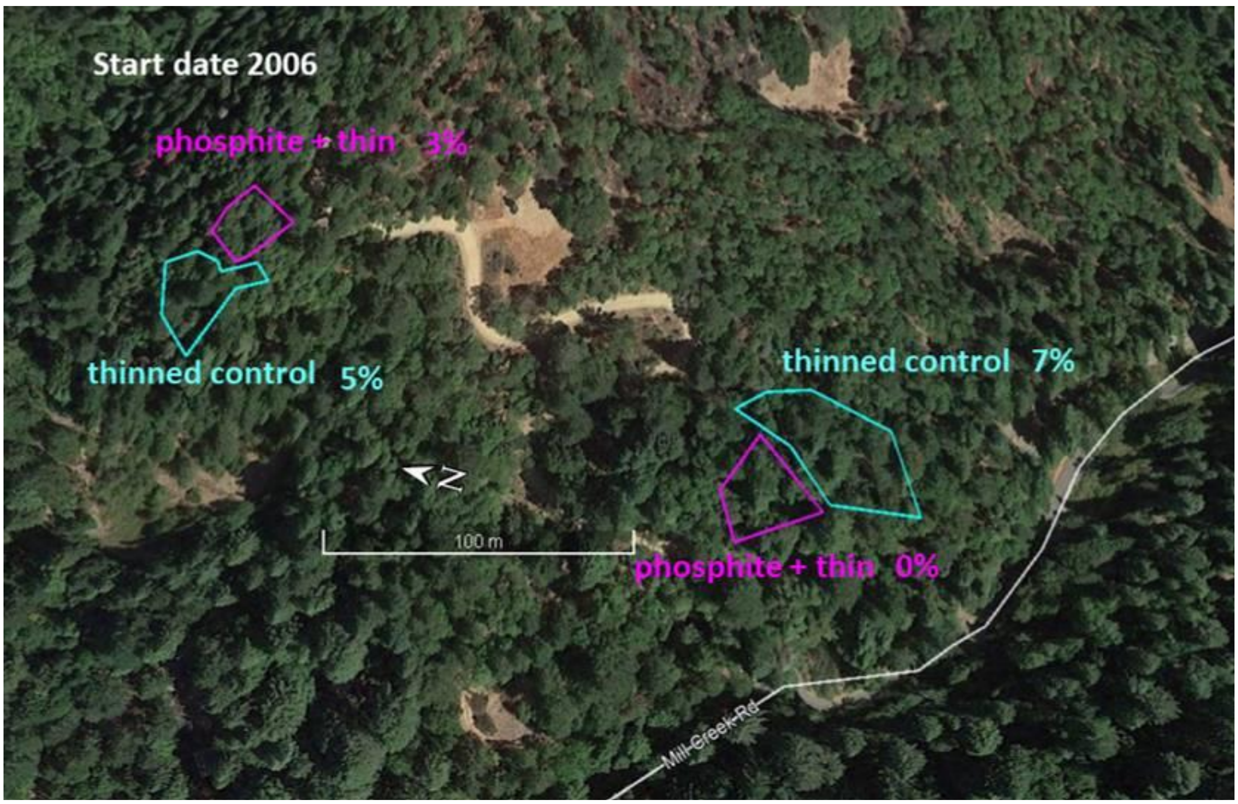


Figure 1-15. Percent SOD infection of tanoaks in phosphite-treated and control FE plots west of Healdsburg as of June 2020. Very few SOD symptoms have been seen in or near these plots. Redwood and Douglas-fir co-occur with tanoak in these plots. Image date September 2018.

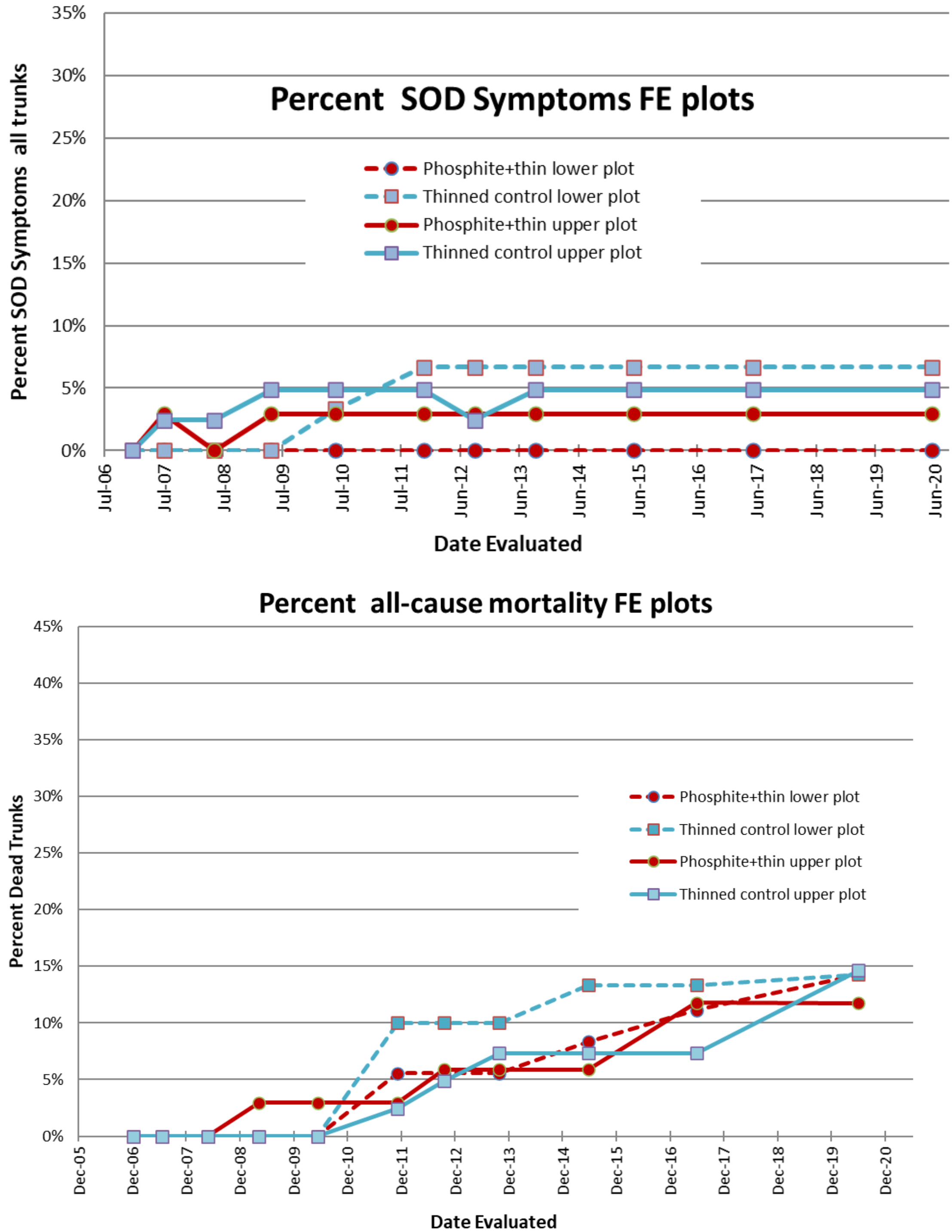


Figure 1-16. Change in percent of tanoak trunks with SOD symptoms (top) and tanoak mortality due to all causes (bottom) over time at FE plots. N=30-41 trunks per plot.

Mortality. Mortality from all causes in the FE plots is shown in Figure 1-16. Three trunks (1 phosphite-treated, 2 untreated) have died due to SOD. Mortality of 7 other trees (3 phosphite-treated, 4 untreated) was related to extensive *Diplodia* cankers.

1.5. Conclusions

One of the primary findings in this study was that the rate at which SOD incidence increases in tanoak stands over time is highly variable between locations and is difficult to predict. Both local and regional climate and rainfall clearly help drive SOD epidemics in tanoak. Most infections develop during wet years, but symptom development in some stems can be delayed for up to several years after a favorable infection period. Few or no infections may develop during drought years, but local rainfall patterns during area-wide drought may allow some infections to develop, so it is not possible to trace every infection to a given year.

Table 1-2 summarizes the results from the 5 plot locations in this study plus an additional study that we conducted during the same time period for a different project (Swiecki and Bernhardt 2017). All studies were conducted using the same application and dosing methodology, small understory tanoaks were removed from treated plots, none of the study plots contained California bay, and phosphite treatment were all started before symptoms were present in treated trees. The studies lasted between 7 and 15.5 years.

The SFPUC (Swiecki and Bernhardt 2017) and El Corte studies used similar overall designs, consisting of a large block of treated tanoaks with multiple control plots distributed around the edges. Both studies used large numbers of trees and had the highest overall stem diameters, with the SFPUC study consisting of primarily large-diameter trees. Although these two study location are only about 7.3 km apart, disease trajectories at the two location were quite different. SOD appeared in the SFPUC plots after 4 years and increased at a rapid rate, with the highest SOD incidence developing in the phosphite-treated plots. In contrast, even though SOD was present in the proximity El Corte plots when they were established, SOD was not detected in the plots for 9 years and increased very slowly except in one control plot. Hence, after 12.5 years, results at this location were inconclusive. Based on the spatial pattern of SOD at this location it was not possible to reliably attribute differences in disease incidence between treated and control plots to a treatment effect.

The remaining study locations were similar in design, utilizing relatively small plots with equal or nearly equal numbers of stems that were matched to the degree possible to provide similar size distributions. However, as noted above, there was no way to match the plots for level of future disease pressure. At FE, disease pressure was uniformly low and no effect of treatment could be detected. At BL, the situation was similar to that at El Corte in that high levels of SOD incidence developed in one control plot (BL nonthinned) after the 2016-17 rains. However, because of the spotty spatial distribution of SOD across the area, high disease levels in this control plot could not be reliably attributed to a treatment effect. SOD incidence did not differ between other control (thinned) and the thinned phosphite-treated plot at BL. At PC, substantial amounts of SOD developed in the phosphite and control plots (both thinned) after the 2016-17 rains, but spatial variation in SOD incidence at this site also makes it impossible to attribute differences between the plots to the phosphite treatment. The SOD incidence in the PC phosphite-treated plot was 18% in 2021, indicating that the treatment was not highly efficacious, especially given that applications had been made for 10 years before the 2016-17 infection period. At SF, disease pressure was high from the start of the study and phosphite treatment clearly did not delay or reduce the final incidence of SOD in the treated plot (Table 1-2).

Table 1-2. Summary data from tanoak SOD management plot locations testing efficacy of bark-applied phosphite using high-bole, diameter-scaled spray applications. Mean control and phosphite-treated tree diameters were nearly identical for all plot sets, and control stem counts were equal to or greater than the number of treated stems. Evaluation of whether SOD incidence was reduced in phosphite treated plots is based on both SOD incidence and spatial distribution of SOD at each location.

	Phosphite-treated plots				**All plots**			
Study plot set	plot size, ha	Number of stems	Mean (std deviation) stem diameter, cm	SOD incidence reduced	Final overall SOD incidence	Years to first SOD symptoms	Total study length, years	Mean % SOD increase per year from first symptoms
SFPUC Skyline	1.35	233	44.0 (16.3)	no	37%	4	9	7.4%
El Corte de Madera	0.36	158	28.3 (13.5)	inconclusive	8%	9	12.5	2.3%
SF	0.05	63	23.6 (20.9)	no	23%	<1	7	3.8%
PC	0.09	75→66	19.3 (10.4)	inconclusive	27%	<1	14	2.1%
BL	0.05	57	16.8 (8.1)	inconclusive	12%	2	15.5	0.9%
FE	0.06	70	19.4 (8.8)	no / inconclusive	4%	1	13.5	0.3%

At 3 locations from this study (El Corte, PC, and SF) as reported above, and in the SFPUC study (significance $p<0.10$), higher disease incidence was seen in larger diameter trees. This effect was not detected at BL where tree diameters were mostly small or at FE where there was little disease. Due to their greater bark thickness and total tree volume, bark-applied phosphite is unlikely to accumulate to effective concentrations in the leaves or phloem of large diameter tanoaks, leading to a lack of efficacy as clearly seen in the SFPUC study. If these large diameter tanoaks are also at greater risk of SOD infection, it seems clear that phosphite application is not an effective SOD management strategy for these trees.

Although phosphite may have some effect in reducing SOD in small diameter tanoaks, we have not been able to confirm this in our studies. Since smaller diameter trees have a lower risk of developing SOD symptoms, at least in some locations, it would be quite difficult to reliably detect a treatment effect unless disease severity in the plot location is uniformly very high. This has not been the case at most of our study locations. In tanoak stands lacking California bay, there were no clear predictors of future SOD severity at our study locations. Even the presence of a symptomatic trees near the plots was not predictive of the future rate of SOD development.

In 2008, we collected leaves from control and phosphite treated plots at SF, BL, PC, and FE locations (n=42 trees, 399 leaves), which were bioassayed for phosphite activity by the Garbelotto lab (UC Berkeley) by inoculating leaf petioles with *P. ramorum* and observing resulting lesion length. We collected leaves from two of the largest diameter trees in each plot and from two trees in the lower quartile of stem diameter. This bioassay failed to show any effect of phosphite in treated trees irrespective of stem diameter or the number of years that the trees had been treated with phosphite (Swiecki and Bernhardt 2008a). At the time, this was largely attributed to an unexplained failure of the bioassay (which had been used successfully in the past). In retrospect, the bioassay could have been functioning as expected and results simply showed that useful levels of phosphite had not been absorbed and translocated to the leaves of treated trees.

Our studies were designed to maximize phosphite efficacy, by using the highest practical doses, applying them where absorption should have been maximized, applying in a preventative fashion, in most cases for multiple years before trees were exposed to *P. ramorum*, and treating blocks of trees to maximize the potential effect of reduced sporulation on treated trees. Nonetheless, some phosphite-treated plots clearly showed no efficacy. SOD symptoms were seen on a few to many trees in all phosphite-treated plots, further indicating that any effect of phosphite treatment on disease development is limited at best. Given that chemical applications require a substantial investment in time and resources that are ongoing indefinitely, levels of efficacy need to be consistently high to justify the time and expense. Our data indicate that this is not the case for bark application of phosphite to tanoak to prevent infection and mortality caused by *P. ramorum.*

Part 2. Effect of California bay removal on SOD incidence in susceptible oak species

These results were reported in our progress report 30 June 2020 when we discontinued further work on these studies. The results have been repeated here with minor editing to consolidate all results related to these contracts in one report.

2.1. Project objectives

1. Monitor effectiveness of localized California bay removal for protecting large, high value oaks

2. Monitor the effectiveness of area-wide California bay removal to protect vulnerable stands of oaks

2.2. Introduction

In contrast to tanoak, SOD infection among susceptible oak species in California is largely a byproduct of the *P. ramorum* foliar disease cycle on California bay. California bay is the most important source of *P. ramorum* spores that cause cankers on SOD-susceptible oaks. 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. 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 5 m of the oak trunk increases (Swiecki and Bernhardt 2008b). Thus, in forests containing both bay and oak, reduction of bay canopy in the vicinity of susceptible oaks provides a means of reducing disease risk.

Foresters have long manipulated stands to favor one species over another. From the spatial relationship between California bay and SOD in susceptible oak species, we inferred that removing California bay from the vicinity of susceptible oak trees should lessen disease pressure and provide a means for controlling disease. 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 alone as an effective long-term means of preventing SOD in tanoak.

California bay removal can be implemented at various scales (Swiecki and Bernhardt 2013). Prescriptions for bay canopy removal as a disease prevention measure need to be tailored to site conditions in order to be practical and cost-efficient. 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 will be most practical when most of the bays in the treatment area are small. This approach was tested in two Midpeninsula Regional Open Space District (MROSD) preserves; at Monte Bello Open Space Preserve to protect Shreve oaks and at Rancho San Antonio OSP to protect coast live oaks from SOD (Table 2-1).

Where this approach is not feasible or desirable, lesser levels of bay removal may still be beneficial, including localized removal of California bay around individual oaks. We used localized California bay removal to protect large coast live oaks at Rancho San Antonio OSP and large canyon live oaks at Russian Ridge and Los Trancos OSPs (Table 2-1) . Because the large bay could not all be removed, we tested combining bay removal with potassium phosphite applications at two of these locations.

Table 2-1. SOD California bay management studies initiated on Midpeninsula Regional Open Space District open space preserves in San Mateo County in 2008 - 2009.

Open Space Preserve	Host species present[1]	Treatment(s) and dates applied	Treated sample size	Control sample size	Notes
Monte Bello—Skid Road trail gate (MB06)	**shreve oak**, canyon live oak	Area-wide bay removal (includes hack and squirt herbicide bay treatments): Dec 2008 /Mar 2009 bay removal, stump treatment, hack and squirt July 2009, May 2010, Dec 2011 bay hack/squirt	95 trunks	84 trunks	Area wide removal over 2.5 ha, controls in adjacent areas
Rancho San Antonio (RSA)—permit lot area	**coast live oak**	Localized bay removal (Nov 2008 and Jan 2015) and phosphite injection: Arborjet injectors Nov 2008, ArborSystems injectors Jan 2011.	9 trunks[2]	61 trunks	Localized bay removal spread over an area of 0.4 ha,
		Localized bay removal (Nov 2008 and Jan 2015) and phosphite trunk spray application: Jan 2009, May 2009, Nov 2009, Nov 2010, Nov 2011, Nov 2012, Nov 2013, Jan 2015, Jan 2016. Dec 2016, Feb 2018.	14 trunks		area-wide removal over 0.3 ha, controls in adjacent areas
		Areawide bay removal only: Nov 2008	42 trunks		
Los Trancos—Near Page Mill Road, Franciscan Loop Trail and Fault Trail	**canyon live oak**, coast live oak	Localized bay removal (Dec 2009, April 2010) and phosphite spray application: Nov 2009, April 2010, Nov 2010, Nov 2012, Nov 2013, Jan 2015, Jan 2016, Dec 2016, Feb 2018. Localized bay removal only: Dec 2009, April 2010, summer 2011	16 trunks 20 trunks	31 trunks	Controls mostly off trail to north of treated areas
Russian Ridge—Near Ancient Oaks Trail	**canyon live oak**	Localized bay removal: Dec 2009, Sep 2010, summer 2011	36 trunks	34 trunks	Controls off trail

[1]Bold font = primary species

[2]One sprayed tree was removed in 11/09. One injected trunk of a multitrunked oak failed in 2009, and the three remaining trunks were switched to spray application in 2010. As a result, the number of injected trunks changed from 13 to 9 and sprayed trunks from 11 to 14.

2.3. Methods

At each study location we tagged trees with numbered aluminum tree tags for monitoring. Individual trunks of multitrunked oaks commonly have different disease outcomes as noted above for tanoak (Swiecki and Bernhardt 2013) 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, *Synanthedon resplendens* (Sesiidae).

Baseline oak health data was collected as described above for tanoak at the start of the study and every one to two years thereafter. 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 500 mW 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 5 m 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.

2.3.1. Area-wide bay removal

Monte Bello Open Space Preserve – Shreve oaks

We tested area-wide bay removal in a stand of Shreve oaks (*Quercus parvula* var. *shrevei*) at the Monte Bello Open Space Preserve in San Mateo County. The treated area (about 2.5 ha) was roughly circular and was dominated by mature Shreve oaks, but included some canyon live oaks. Within the treated area, California bay occurred mostly as scattered, small diameter understory seedling and saplings and a small a number of large, mostly multitrunked trees. Prior to bay removal, we tagged 73 oaks for monitoring: 58 Shreve oak trees (73 trunks, mean DBH 36 cm, range 11-81 cm), and 15 canyon live oak trees (18 trunks, mean DBH 43 cm, range 11-102 cm).

We also tagged 74 oaks located beyond the edges of the treated area to serve as controls: 63 Shreve oak (76 trunks, mean DBH 33 cm, range 12-80 cm), and 11 canyon live oak trees (13 trunks, mean DBH 37 cm, range 6-92 cm). For all tagged trunks, we recorded baseline health data, oak-bay clearance, and bay cover within 2.5 and 5 m of each trunk as described above for coast live oaks. A few trunks in each treatment already had SOD symptoms.

Removal of California bay trees, saplings, and seedlings was initiated in December 2008 and completed in March 2009. Clearing was done by MROSD staff and CCC crews. Stumps of removed trees were immediately treated with glyphosate (20.5% a.i. solution) to suppress resprouting. Felled material was left on site after being cut in small pieces and dispersed away from oak trunks (lopped and scattered). In March 2009, 13 California bays that were too large to fell with the available crew were treated with glyphosate by making downward-angled cuts around the circumference of the trunks using a hatchet and immediately spraying glyphosate (20.5% a.i. solution) into the cuts using a backpack sprayer (known as frill girdle or hack-and-squirt application). Three large bays in the treated area were inadvertently skipped in March and were treated by frill application in July 2009 and again in May 2010 and December 2011. California bay trees along a creek that bordered the treated area were not removed.

Rancho San Antonio – coast live oaks

Both area-wide and localized bay removal were tested at this location (Figure 2-1). Bay removal was initiated in 2008. Area-wide bay removal covered about 0.3 ha and we monitored 42 trunks in the treated area (Table 2-1). We monitored 61 trunks in nontreated control areas around the treated areas (Figure 2-1, Table 2-1).

2.3.2. Localized bay removal

Rancho San Antonio - coast live oaks

Large diameter coast live oaks in and near the Permit Lot were treated with localized California bay removal in 2008 (Table 2-1). All small understory bays near the oaks were removed, and larger bays were removed where feasible. Some very large bay trees that were deemed too large to remove remained near the Permit Lot, although limbs were removed in some cases to increase bay-oak clearance and reduce overall bay density. Eleven large diameter coast live oaks near the Permit Lot were also treated with annual trunk spray phosphite application between 2008 and 2018, using the same methods used at El Corte de Madera OSP (described in Part 1 of this report). Ten trees that were close to the creek were treated with phosphite injection in November 2008 using the Arborjet "Tree I.V." injection system. We used the UC Berkeley/Agri-Fos rates (1.75 ml of non-diluted Agri-Fos / inch stem diameter). We used Agri-Fos diluted at 1:5 with water rather than the 1:2 dilution advised by UC Berkeley for the Chem-jet injectors they were using at that point. We also used the Arborjet label spacing for the injection holes, which corresponds to about one injection hole per 9 to 10 inches of stem circumference, rather than the 6 inch spacing used with the Chemjet injectors.

In January 2011, we retreated the injected trees using an alternative type of tree injector from ArborSystems (arborsystems.com). They provided a Wedgle Direct-Inject Quick Connect™ injection system with Portle® injection tips. This system does not require drilling into the wood. The Portle® tips have a number of small holes along the sides of the tip which allow for chemical delivery into the inner bark, near the cambium. After driving the tip into the tree with a slide-hammer device, the injector is connected to the tip. The desired amount of chemical is pumped through the injector by squeezing the handles. The ArborSystems Portle injector tips have a built-in check valve that prevents chemical from leaking back out of the tip until it is absorbed by the tree. Once the chemical is absorbed the tips can be pulled from the tree.

Each pump of the injector nominally delivers 1 ml of liquid. We used a graduated cylinder to verify that the target amount of chemical was delivered through each tip. This setup also allowed us to use a 1:1 dilution of Agrifos, rather than the full strength solution (620 g potassium phosphite/L), which is used in the ArborSystems prepackaged formulation. We preferred to use the 1:1 dilution to reduce the potential for phytotoxicity. For the Arborjet Tree I.V. injections, Agri-fos is diluted 1:5 with water and for the Chemjet injectors a 1:2 dilution is used. The ArborSystems injectors were used to treat only those trees previously injected using the ArborJet Tree I.V. injectors. We injected 6 ml of 1:1 diluted Agri-fos solution at each injection point. The overall applied dose was 1.75 ml non-diluted Agri-fos (45.8% potassium phosphite) per inch DBH, which is the same rate as used with the Chemjet injectors. This required a spacing of 12-14 cm between injection points.

Los Trancos OSP – canyon live oaks

Initial treatments began in December 2009 (Table 2-1.). California bay removal was used to protect 36 large diameter canyon live oaks along Franciscan Loop and Fault Trails. In addition, phosphite trunk spray applications were also started in 2009 on 16 of the largest trees to potentially provide an additional measure of protection. Trees were treated using the same methods as were employed at El Corte de Madera OSP (Part 1 of this report). Control trees were selected in off-trail areas to the north of the treated trees. At the start of the study, it was difficult to find canyon live oaks that were close to California bay that did not already have SOD symptoms. Consequently, we included a number of trees with SOD symptoms at the start of the study. Eight of the 36 treated trees and 10 of the 31 control trees had SOD symptoms at the start of the study. The last phosphite treatment was applied in February 2018. Disease progress was evaluated annually through 2020.

Russian Ridge OSP – canyon live oaks

Initial treatments began in December 2009. California bay removal was used to protect 36 large diameter canyon live oaks along the Ancient Oaks trail. Over the next few years, additional clearing occurred which increased clearance between oaks and nearby bay (Table 2-1.). As at Los Trancos OSP, some oaks were symptomatic at study start. Disease progress was evaluated annually through 2020.

2.4. Results

2.4.1. Rancho San Antonio Open Space Preserve (RSA) – coast live oak – localized and area-wide bay removal

Both area-wide and localized bay removal were tested at this location (Figure 2-1). Large diameter coast live oaks in and near the Permit Lot covering about were treated with localized California bay removal in 2008. All small understory bays near the oaks were removed, and larger bays were removed where feasible. Some very large bay trees that were deemed too large to remove remain near the Permit Lot, although limbs were removed in some cases to increase bay-oak clearance and reduce overall bay density. In some cases, these tall bay canopies are very close to or overtop nearby coast live oaks. However, conditions for bay leaf infection in the tall canopies are likely to be less suitable for *P. ramorum* (more light, higher temperatures, lower humidity, shorter leaf wetness period) than in understory bay leaves. Hence, removing dense bay understory that was present at the start of the study greatly reduced local *P. ramorum* inoculum density even though not all overstory bays were removed.

Eleven large diameter coast live oaks near the Permit Lot were also treated with annual trunk spray phosphite application between 2008 and 2018. As described below, because disease pressure at this site was low and California bay removal was very effective, it did not appear that continuing to treat the trees with phosphite was likely to provide any additional disease suppression. MROSD discontinued phosphite application at this location after the February 2018 treatment.

Ten trees that were close to the creek were treated with phosphite injection in November 2008 and January 2011. The injection treatments were discontinued when it became apparent that the labeled rate caused phytotoxicity at the injection points. Given the amount of phytotoxicity caused at the original recommended rates, it is doubtful that much, if any, of the injected phosphite was translocated beyond the damaged xylem tissues.

Through the course of this study, disease pressure at RSA was much lower than at the other MROSD preserves where SOD disease management treatments were tested. RSA is more inland and presumably warmer and drier than the SOD plots in preserves along Skyline Ridge. This appears to have limited the persistence of *P. ramorum* over the extended drought of 2011-2016 and reduced overall disease pressure.

No new SOD was confirmed in plots in the final June 2020 evaluations. We rated one additional coast live oak as SOD positive based on symptoms, even though our isolation was negative. This large coast live oak is overtopped and surrounded by bay. The chipped canker appearance was typical of those caused by SOD, but was somewhat dry. Dry SOD cankers typically have a low success rate for direct isolations. Symptoms of *P. ramorum* infection on California bay foliage were practically nonexistent at this location in June 2020, suggesting that most of the infected leaves observed in the previous evaluation (August 2018) had fallen and few or no new bay leaf infections had developed in the relatively dry 2018-19 and 2019-20 rainy seasons.

Because overall SOD incidence was very low and samples sizes in the individual treatments are relatively small, the differences in disease incidence between the control and each of the three treatments were not large enough to be significantly different. If all three treatment groups are combined, under the assumption that bay removal provided the overall treatment effects and the two different phosphite treatments were unlikely to have had separate effect, the difference in SOD incidence in 2020 between all treated (1.6%) and control (12.9%) stems was significant at P=0.0324 (Fisher's exact test). The incidence of SOD among oak trunks for the different study treatments over the course of the study is shown in Figure 2-2.

A pulse of new *P. ramorum* infections was seen after the extreme rainfall of the winter of 2016-2017, primarily in the control plots. Control trees that developed new SOD symptoms had bay foliage overhanging their main trunks and had 50 to 100% bay foliage cover within a radius of 2.5 m around their trunks. This represents a high level of potential exposure to *P. ramorum* inoculum from bay foliage. Through June 2020, three of eight *P. ramorum*-infected trees died in the control plots. None of the trees with SOD symptoms in the treated plots died though June 2020.

One new SOD-infected coast live oak was seen in the area-wide bay removal plot across the creek from the permit lot during the 2018 disease evaluations. This tree is at the toe of a steep slope with large California bay trees. The nearest bay foliage is 13.8 m from the oak trunk. This is an anomalous infection of a tree which is far enough from bay to have a low infection risk. Such anomalous infections are seen occasionally. However, because the slope with the bay is relatively steep, aerial spore dispersal could be further than typical because wind-blown spores would remain airborne longer. In addition, downhill flow of rainfall runoff could have carried *P. ramorum* spores and infected bay leaves downslope, where they could have contacted the base of this tree.

The only other new SOD infection recorded for the area-wide bay removal treatment was recorded in 2014. That infection resulted from expansion of a SOD canker on a large tree with a low fork being monitored as two separate trunks. The canker was present in 2008 at study start. It expanded from one side of the tree to the other side of the tree, and thus did not represent a new infection from aerially-dispersed inoculum. Therefore this trunk infection was not counted in the analysis of the effect of bay removal.

Overall, six monitored trees died due to factors other than SOD, three in treated areas and three in control areas. Several trees experienced root and crown failures due to decay by true fungi. At least one failure near the creek bank also involved soil failure during wet soil conditions.

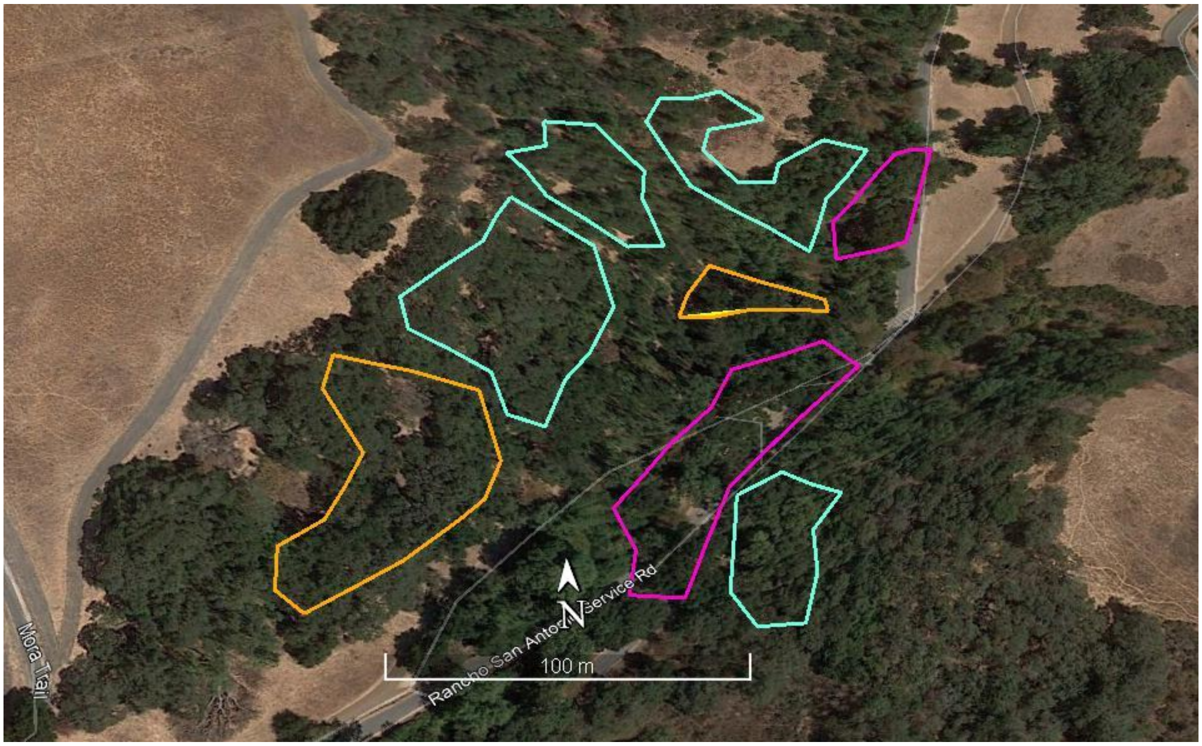


Figure 2-1. Rancho San Antonio Open Space Preserve coast live oak sudden oak death (SOD) treatments study plots. Blue polygons – areas where control trees were monitored (N=62), magenta polygons – localized California bay removal and phosphite treatments (trunk spray N=11, injection N=10, total area 0.4 ha), orange polygons – area wide bay removal (N=41, total area 0.3 ha).

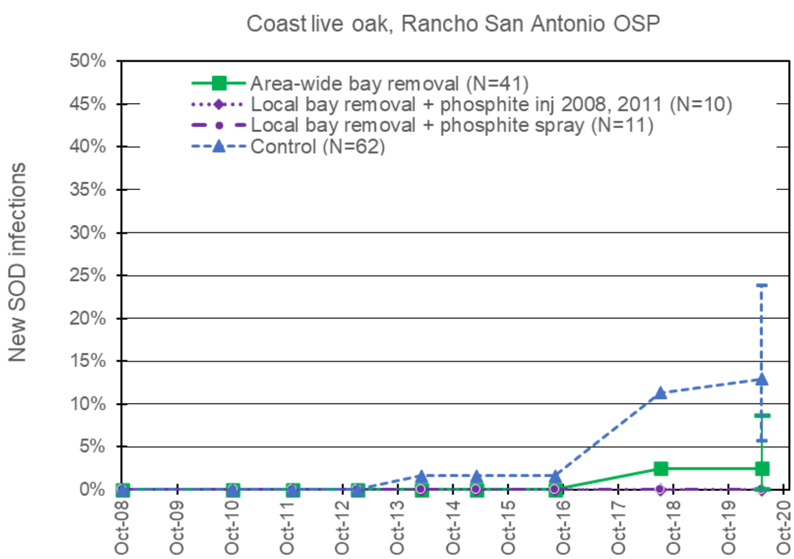


Figure 2-2. Increase in new SOD infections over time at Rancho San Antonio Open Space Preserve coast live oak sudden oak death (SOD) treatments study plots. Error bars are exact binomial 95% confidence limits for area-wide bay removal without phosphite and control treatments.

2.4.2. Los Trancos Open Space Preserve – canyon live oak – localized bay removal

Of all the SOD-susceptible oak species, canyon live oak is the most difficult in which to observe and diagnose SOD symptoms. Cankers are often cryptic with no bleeding evident. Even when bleeding is present it can be difficult to find the leading edge of the canker where a successful isolation can be made. In addition, although bleeding may occur shortly after infection, the amount of bleeding is generally small and may not persist to be observed in subsequent years. If the cankers continue to expand, they are eventually attacked by ambrosia beetles and commonly develop sporulation of *Annulohypoxylon thouarsianium*. However, when these late-stage symptoms are the first indication of infection, it is usually not possible to isolate *P. ramorum* from the cankers. As a result, there can be considerable uncertainty as to when *P. ramorum* infections occurred. This can affect the interpretation of treatment effects because some trees that appeared to be asymptomatic before bay removal treatments were imposed may actually have been cryptically infected. Hence, some of the symptoms that develop after treatment do not reflect the efficacy of the treatment.

The plot layout at Los Trancos Open Space Preserve is shown in Figure 2-3. At the start of the study, it was difficult to find canyon live oaks that were close to California bay that did not already have SOD symptoms. Consequently, we included a number of trees with SOD symptoms at the start of the study. These were evaluated for disease progress, along with asymptomatic trees. Eight of the 36 treated trees and 10 of the 31 control trees had SOD symptoms at the start of the study. The 36 treated trees were treated with localized bay removal. Of these, 16 large-diameter trees located along major trails were also treated with phosphite by trunk spray application. The last phosphite treatment was applied in February 2018; phosphite treatment was subsequently discontinued. By this time, no new disease had been seen in any of the trees treated with local California bay removal only, and it seemed unlikely that continuing phosphite treatment would demonstrably improve disease suppression.

Figure 2-3. Los Trancos study plots. Localized bay removal was conducted around groups of canyon live oaks in the area bounded by the red line. Red icons represent control trees. Treated trees: green icons=localized bay removal at study start in 2009; blue=localized bay removal in 2011; purple icons=localized bay removal at study start + phosphite treated though 2018.

Final evaluations of the trees in this study trees were made in June 2020. None of the 18 trees that were infected at the start of the study in 2010 had died by June 2020, although one of the initially-infected controls had >97% canopy dieback and was nearly dead. These data show that decline and mortality of SOD-affected canyon live oaks can be very slow, especially if the trees are large. Overall, SOD-related decline appears to be slower in canyon live oak than in coast live oak. Furthermore, we have previously shown that coast live oak is much more likely to fail within a few years after late SOD infection symptoms (ambrosia beetle boring and/or *A. thouarsianum* sporulation) appear. Our observations indicate that it takes much longer before failure potential in SOD-infected canyon live oak increases substantially.

Prior to June 2020, no new SOD infections had been seen among any of the 28 initially asymptomatic trees treated with local California bay removal (Figure 2-4). In the June 2020 evaluation, we found an apparent SOD canker in a previously asymptomatic treated tree (tag 1215). This tree had been treated by localized bay removal and had also been treated with potassium phosphite bark spray through February 2018. This tree was displaying late symptoms of infection; our isolation for *P. ramorum* was negative. The most likely explanation for this symptomatic tree is that it became cryptically infected during the extremely wet winter of 2016-2017 when disease pressure was unusually high, and infection only now was advanced enough to be apparent. The minimum distance measured between bay foliage and the trunk was 11.1 m, but levels of bay foliar infection at this location are typically high, increasing the likelihood of some longer-range aerial movement. Another possibility is that this trailside tree was climbed by some trail users. Absence of moss on the lowest branch and a bark wound near that branch give credence to this hypothesis. It is possible that inoculum was transported to the trunk of this tree via contaminated footwear during the wet season. As noted below, this mode of inoculum transport has been suspected for at least two large canyon live oaks at Russian Ridge. It is also noteworthy that annual treatment of this tree with phosphite bark spray through Feb 2018 did not prevent the infection that most likely occurred in the winter of 2016-2017.

Among untreated canyon live oak controls, 5 of the 21 initially asymptomatic trees developed symptoms by June 2019. No additional symptomatic trees were seen among these controls in June 2020. None of the infected controls had died as of June 2020, although one that initially showed SOD symptoms in 2012 was in severe decline with more than 80% canopy dieback. Although disease incidence among initially asymptomatic trees at this location was higher in controls (23.8%) than in treated (3.6%) trees, the significance level of this difference is only p=0.0716 (Fisher's exact test) due to the small overall sample size.

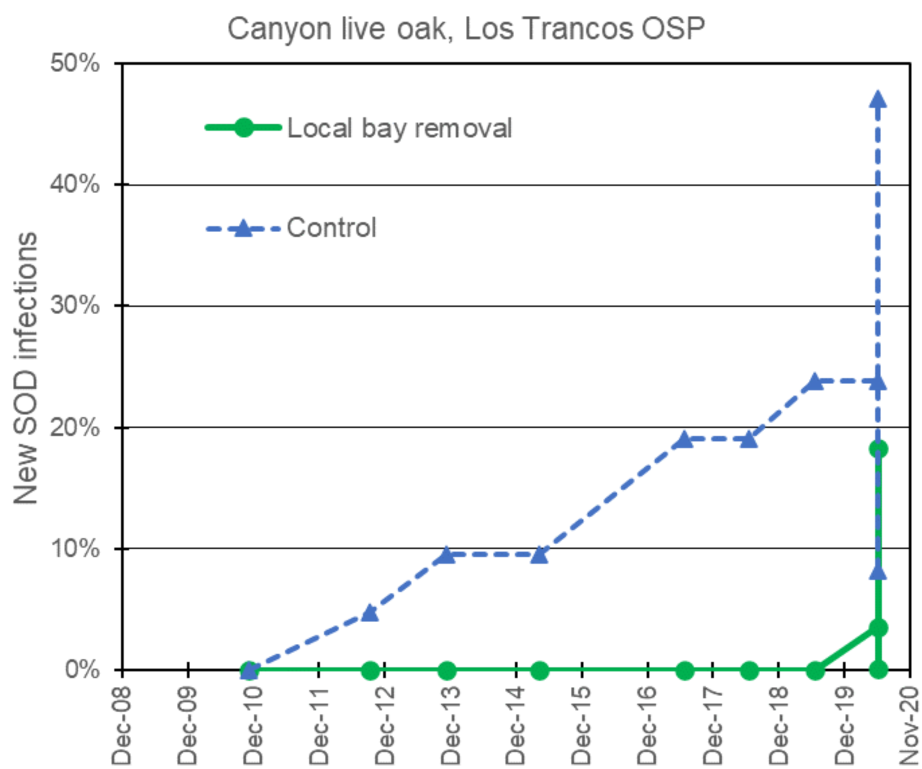


Figure 2-4. Increase in SOD incidence over time at Los Trancos OSP among canyon live oaks that were asymptomatic in 2010. One possible new infection was seen among controls (N=21), discussed in text. Local bay removal (N=28) includes all trees with local bay removal; 12 of these were also treated with phosphite from 2010 through 2018. Error bars are exact binomial 95% confidence limits.

2.4.3. Russian Ridge Open Space Preserve – canyon live oak – localized bay removal

At Russian Ridge Open Space Preserve, targeted bay removal was evaluated to protect a population of very large canyon live oaks along and near the Ancient Oaks trail (Figure 2-5). No phosphite applications were used at this location. Bay removal occurred in December 2009, September 2010, and summer 2011, generally localized around individual trees or groups of trees close to the trail. Control trees were located further from the trail, beyond the bay removal areas.

Figure 2-5. Russian Ridge Open Space Preserve canyon live oak bay removal study. White icons are control trees, green icons are treated trees.

Localized bay removal was very effective in preventing new SOD cankers among the large canyon live oaks (Figure 2-6). By May 2014, SOD symptoms appeared in two of 34 treated (bay removal) trunks of a large multitrunked canyon live oak that was initially asymptomatic. Given the cryptic nature of *P. ramorum* cankers in canyon live oak, it is likely that these infections occurred before the start of the study. Since that time, no new infections had been seen among initially asymptomatic oaks treated with local bay removal until June 2020. We noted a small area with bleeding on tree 493 (Figure 2-7), which is one of the largest and most iconic trees. The bleeding is on the top of one of its two trunks, and based on wearing of the moss and bark, appears to be associated with tree climbing. We did not chip the bark to look at the appearance of the canker or attempt an isolation due to the position of the canker on the tree. Distance between bay foliage and the trunk was 7.5 m. Bay foliar infection levels at this location are generally high.

In contrast, among initially asymptomatic control canyon live oaks (n=27), three trunks developed SOD symptoms by 2014 and the number of newly symptomatic trees continued to increase to 12 (44%) in 2019. As at other locations, a steep increase in SOD incidence was seen after the wet 2016-2017 rainy season. No newly symptomatic control trees were seen in June 2020. Among initially asymptomatic trunks, the difference in SOD incidence between the bay removal treatment (9%) and controls (44%) is highly significant (P=0.0005, Fisher's exact test). This is a conservative estimate of the difference because at least some of the infections of the treated trees likely predate the treatment.

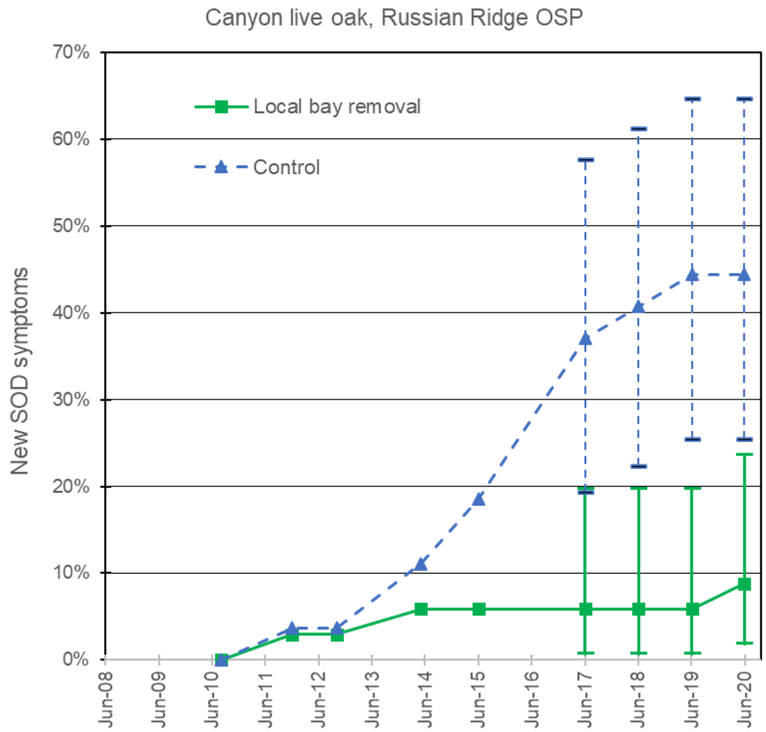


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Figure 2-6. SOD incidence (2010-2020) in initially asymptomatic canyon live oaks at Russian Ridge Open Space Preserve treated by local California bay removal (N=34, solid line, square icons) and in untreated control areas (N=27, broken line, triangle icons). Error bars are 95% exact binomial confidence intervals.


Figure 2-7. Tree 493, newly symptomatic June 2020 with suspected SOD canker on upper surface between trunks.

Initially symptomatic trees. As at other locations, a few trees that were symptomatic at the start of the study were included for monitoring. Among the 6 initially symptomatic controls, one has died. This tree had a large SOD canker and had failed at the base in 2017 and remained green initially, but was dead by June 2019. Most others have shown an increase in canker girdling rating since 2010, but several show callusing around old cankers. All four of the initially symptomatic canyon live oak trunks included in the bay removal areas are still alive, though canker girdling has increased on three of these. The tree showing the greatest canker expansion is a tree 484, a very large tree (270 cm DBH) located directly on the trail that is very commonly climbed upon by trail users. It appears that additional infections have been initiated through this activity from inoculum deposited from mud and debris on climbers' shoes and wounds created on the bark. The canopy of this tree has died back and thinned noticeably as the amount of girdling has increased.

Mortality. Two of the monitored trees in the control area died when they were knocked down by the failure of nearby adjacent dead trees in 2014 and 2015. These and the SOD-killed and failed control noted above are the only study trees that have died to date at this study location.

Bay foliar symptoms. *P. ramorum* infection symptoms on bay leaves were not prevalent in June 2020, indicating disease pressure was low. In June 2019, bay foliage showed very heavy *P.*

ramorum infection levels, indicating strong disease pressure for the 2018-19 wet season, but possibly not as high as seen for the 2016-17 wet season.

2.4.4. Monte Bello Open Space Preserve – Shreve oaks – Area wide bay removal

At the Monte Bello Open Space Preserve, area-wide California bay removal was undertaken to protect a unique stand of Shreve oaks. The plot layout is shown in Figure 2-8. Overstory and understory California bay was removed from a large central treated area and surrounding areas without bay removal were designated control areas. We tagged and periodically evaluated 84 trees in the control area and 95 trees in the treated area. The final evaluation of tagged trees for SOD symptoms was in June 2020.

SOD incidence in the controls has been significantly higher than in the area-wide bay removal plot since the 2013 evaluations. Starting with the 2018 evaluation and continuing into 2019, a large increase in SOD incidence was observed in untreated control trees (Figure 2-9), doubtless related to favorable conditions for disease spread and infection associated with the record rainfall in winter of 2016-17. This increased the difference in SOD incidence between the treated bay removal plot and the controls. The rate of disease increase tapered off in 2020, in part due to the dry conditions that occurred in winter and spring of 2020.

In 2019, we confirmed that one of the tagged Shreve oaks in the bay removal treatment area had developed a basal *P. ramorum* canker. Although no California bay trees or saplings were observed within 20 m of this tree, it was located in a very dense patch of poison oak, a *P. ramorum* host that supports sporulation. We have previously seen rare situations where poison oak climbing in oak canopies was the only apparent source of *P. ramorum* inoculum, but had not previously seen a situation where *P. ramorum* infection appeared to be associated with shrubby (up to 1 m) stands of poison oak around susceptible oaks.

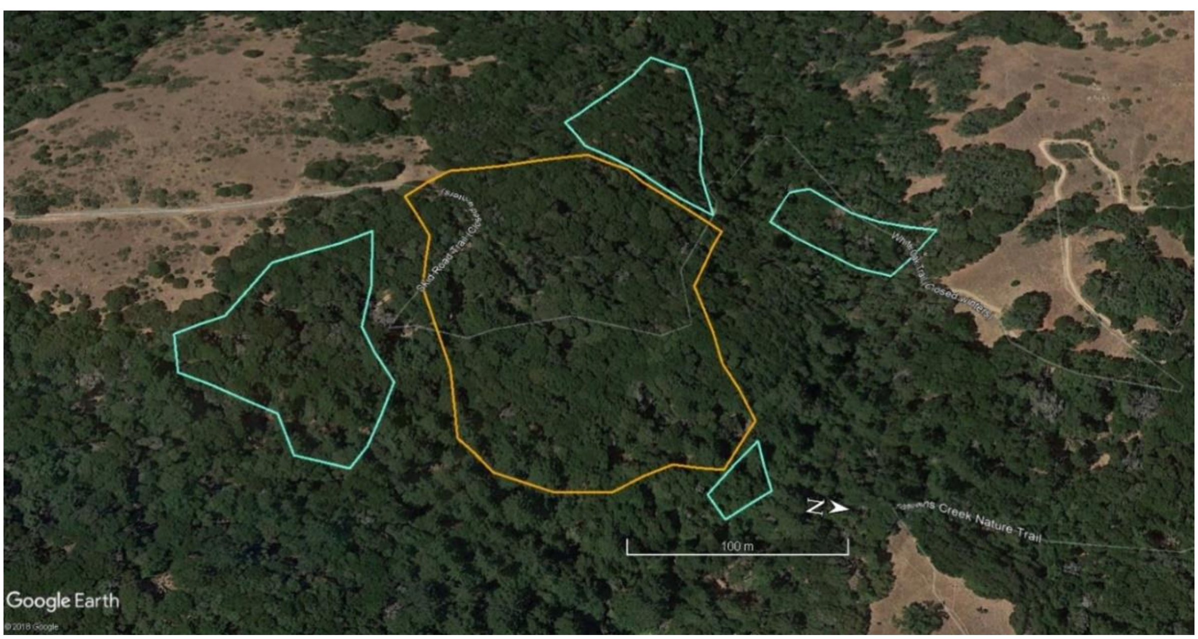

Figure 2-8. Area-wide bay removal study plots at Monte Bello Open Space Preserve. The orange polygon indicates where bay removal area occurred, 2.5 ha. Cyan polygons show where monitored control trees are located.

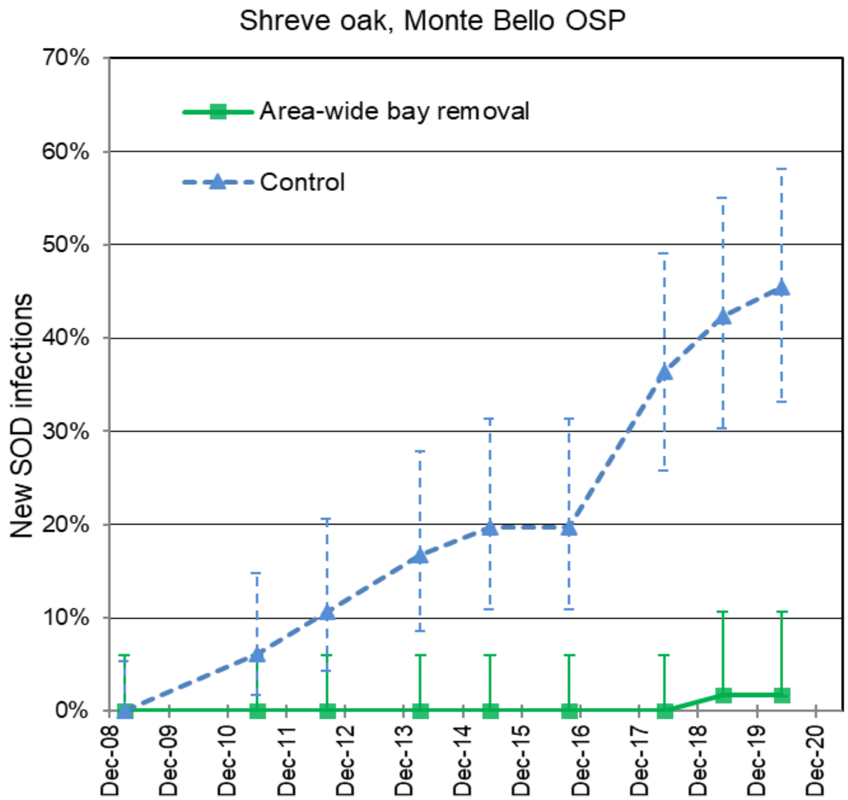

Figure 2-9. SOD incidence among initially asymptomatic Shreve oaks in area-wide bay removal and untreated control areas at Monte Bello Open Space Preserve. N=66 control trunks and 60 treated trunks. Error bars are 95% exact binomial confidence intervals.

Mortality. Among tagged Shreve oaks that were asymptomatic at the study start, five control trees and no treated trees have died due to *P. ramorum* cankers. Three additional control trees and five trees in the bay removal area have died from causes other than SOD.

Canyon live oaks. At this location, a few canyon live oaks that occur within control (10 trees) and bay removal (18 trees) areas were tagged and evaluated. Disease symptoms consistent with SOD developed on one large (DBH = 91 cm) tree in the treated area in 2013. Given the cryptic nature of SOD symptoms on canyon live oak and the long latent period between infection and symptom expression, this infection could have been initiated before the bay removal treatment was conducted. Disease has continued to advance in this tree over time. Symptoms consistent with SOD have not developed on other tagged canyon live oaks at Monte Bello.

2.5. Conclusions

These studies show that over an extended time period (2008 to 2020) California bay removal was a highly effective control measure to protect susceptible oaks from infection by *P. ramorum*. Even in locations where differences in disease development were not significant, lower SOD incidence was consistently observed in trees treated with bay removal. In tanoak stands lacking bay, the spatial distribution of SOD hotspots appears to be largely stochasitic and may be related to long range inoculum dispersal events, variation in sporulation potential between individual trees, and/ or other factors that are largely cryptic. In contrast, SOD risk in oak is very strongly related to the proximity of susceptible oaks to local inoculum sources, the most important of which is California bay. Hence, the interpretation of differences between control and treated trees in bay removal is straightforward. Within a given location, once a sufficient number of new infections develop on control oaks that are close to California bay trees, we can have high confidence that a lack of infections in matched oaks with large bay clearances created by bay removal is related to the treatment.

The few infections that developed among oaks treated with local or area-wide bay removal were mostly related to unusual situations, including the possible transmission by open space users climbing on trees. Direct deposition of inoculum in soil or plant debris from contaminated footwear (Davidson et al. 2005) on trunks may pose an infection risk to oaks that are too far from bay foliage to be threatened by aerial spore dispersal. Bark wounds created by climbing might also enhance infection. Public education and signage may be needed to inform trail users that this common activity may pose a lethal threat to old and highly prized oaks.

Some specialized situations noted above, such as slopes with bay above oaks and heavy poison oak density near oak trunks, may only come into play when disease pressure is especially high. In extremely rainy winters, such as the 2016-2017 rainy season, *P. ramorum* sporulation levels can be very high and spore movement may allow infections to occur at greater California bay-oak clearances than is typical and may lead to significant levels of sporulation on hosts such as poison oak. These less typical risk situations should be considered when conducting bay removal treatments, especially in areas that are especially conducive for *P. ramorum*. In areas where bay densities are high, localized bay removal needs to extend as far as practical away from target oaks to achieve a high level of protection. Where large clearances cannot be achieved, some breakthrough infections may occur in rainy years.

Over the course of the study, during our data collection visits we used hand saws and pruning shears to cut back bay spouts or branches that began to infringe on bay-oak clearance within treated areas, especially where localized bay removal had been used. To maintain treatment effectiveness over time, follow-up treatments need to be conducted on a regular basis to suppress bay sprouts, branch growth toward oaks, and establishment of new bay seedlings near oaks. The retreatment interval will vary depending on the bay density and growth rate, and the degree to which bay spouts are suppressed by deer browsing, soil moisture availability, and other factors. Initial monitoring should be done in the first year after bay removal treatments to locate material that may have been missed or has resprouted vigorously. If sprouting is well controlled due to herbicide application and/or browsing by deer, inspections can be repeated every 2 to 3 years with spot treatment as needed, with wider retreatment at intervals between 5 and 10 years.

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