Management of *Phytophthora ramorum* in tanoak and oak stands

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Progress report #6 - 6/30/09

This progress report covers the period January-June 2009. Major tasks completed over the last 6 months include:

Tanoak phosphite treatment study
- reevaluated disease status in all tanoak plots
- resprayed Agri-Fos tanoak plots
- collected samples to test *P. ramorum* inoculum levels in Agri-Fos treated and control tanoak plots. Assays were conducted by Dr. Elizabeth Fitchner in the Rizzo lab.

Coast live oak and California black oak bay removal study
- renewed permits in preparation for plot re-evalution
- reevaluated disease status and bay regrowth in study plots.

A more detailed account of project activities is described below.

Introduction

The objective of this study is to test methods for managing *Phytophthora ramorum* canker in tanoak and oak stands. Because disease epidemiology differs between different canker hosts, we are testing different control strategies in tanoaks and susceptible oaks. For tanoak, we are studying the use of potassium phosphite (Agri-fos®) applied as a protectant spray. For coast live oak and California black oak, we are testing whether localized bay removal and/or pruning will reduce the risk of *P. ramorum* infection to acceptably low levels. The tanoak / phosphite portion of the study is being conducted in collaboration with Matteo Garbelotto and Yana Valachovic. The long term plots we have established for this project are located in Napa, Solano, and Sonoma counties.

Objective 1. Protection of tanoak stands using bark-band application of phosphite and understory thinning.

The Phytosphere tanoak/phosphite plots are located in two geographic areas. All plots are in spatially grouped sets, with each set containing one Agri-Fos treated plot and one or two paired control plots (Table 1-1). Three sets of plots are located in two rural subdivisions in northwestern Sonoma County (Seaview Ranch and Gualala Ranch), which are located about midway between Plantation (near Salt Point State Park) and Cazadero. The remaining two plot sets are on a property along Mill Creek, west of Healdsburg.

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Plots at the SF and BL sites were established in cooperation with the Kashia Band of Pomo Indians of Stewarts Point Rancheria, and most of the activities associated with those locations is currently conducted under a separate contract with the Kashia, with funding provided by USDA-FS State and Private Forestry.

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

Study site	Locality	Plots	Agri-fos applications	Notes
SF	Seaview Ranch, Creighton Ridge area	1 Agri-Fos treated+thinned 1 thinned control 1 nonthinned control	Dec 2005 May 2006 May 2007 May 2008 May 2009	Plots initially established 2005 (Kashia Band of Pomo Indians cooperating).
BL	Gualala Ranch Creighton Ridge area	1 Agri-Fos treated+thinned 1 thinned control 1 nonthinned control	Dec 2005 May 2006 May 2007 May 2008 May 2009	Plots initially established 2005 (Kashia Band of Pomo Indians cooperating).
PC	Gualala Ranch Creighton Ridge area	1 Agri-Fos treated 1 control	Jan 2007 May 2007 May 2008 May 2009	Understory tanoak mostly pre-thinned by landowner. Some minor additional thinning was conducted in treated and nontreated plots.
FE	Mill Creek Road, Healdsburg	2 Agri-Fos treated 2 control	Feb 2007 May 2007 May 2008 May 2009	Understory tanoak mostly pre-thinned by landowner. Some minor additional thinning was conducted in treated and nontreated plots

The 12 plots have 656 tanoak stems which are being individually tracked. The number of stems in each plot is shown in Table 1-2. We assessed the disease status of all stems in plots in May 2009. Stems in Agri-Fos treated plots were assessed prior to being resprayed. Dead stems and stems that were nearly dead were omitted from the list of stems to be sprayed. Because a known amount of spray solution is applied to each stem, we recalculated the amount of spray needed for each plot based on the number of live stems.

All treated plots were retreated with Agri-Fos in May 2009. All plots were treated using the 4 gal (15 L) ShurFlo Propack electric backpack sprayer mounted on a modified mountain bike. As in previous applications, we banded the spray high on the stem (3 to 6 m height) using a long telescoping spray wand to maximize phosphite uptake. Methods used to calculate the spray volume for each stem were as described in our December 2007 progress report. By using a specific diameter-related spray volume on each stem, we should minimize the variation in the applied phosphite dose. In previous years, we have been able to coordinate with Kashia tribal personnel to jointly spray the plot with the most difficult access (SF). However, Kashia personnel were not available within the time window for the application this year so we rented a SUV and sprayed this plot by ourselves.

Inoculum monitoring in plots

During the spring of 2007, we monitored inoculum production within the plots using buckets containing floating bay leaf baits as described in the December 2007 report. This method is only likely to detect *P. ramorum* inoculum if substantial amounts of rainfall occur while the buckets and baits are in place. Although small amounts of rain fell during the baiting period in spring 2007, no *P. ramorum* was detected with the bay leaf baits.

Due to drought conditions in 2008 we anticipated that there would not be enough rainfall for the floating bay leaf baiting technique to succeed. As an alternative method for assessing inoculum production in the plots, we turned to a soil baiting method that Elizabeth Fitchner from Dave Rizzo's lab at UC Davis had developed. The method uses rhododendron leaf disks to detect *P. ramorum* in flooded soil samples. After the baiting period, all leaf discs are plated onto PARP-hymexazol media, whether or not they are showing symptoms. Dr. Fitchner agreed to conduct the soil baiting for soil samples from our plots, as well as the Garbelotto and Valachovic plots. The soil from all of our plots tested negative for *P. ramorum*. As we have confirmed *P. ramorum* infections in many of the plots, lack of positive results appears to be related to the dry conditions in spring 2008. Low rainfall clearly reduced inoculum production in spring 2008, so inoculum levels in the soil would have been very low. Furthermore, the dry soil and warm temperatures may have reduced the survival of any inoculum in the soil.

In 2009, another dry year, we again used soil baiting to test of inoculum in the plots. We collected soil samples from all plots in May, prior to the Agri-fos applications. Elizabeth Fitchner used rhododendron leaf baiting as in 2008. All samples were negative.

Sand spore trap

The inoculum monitoring methods used to date are especially problematic when inoculum production is low and when rainfall events that may result in inoculum production are widely scattered. We were interested in developing a method for monitoring inoculum production that could take advantage of *P. ramorum*'s extended survival in soil but could be more sensitive to lower inoculum levels and less subject to variance associated with soil differences from place to place. In consultation with Dr. Elizabeth Fitchner, we developed a spore trap and built six prototypes for field testing.

The spore trap uses a wide plastic tray that collects rainfall and funnels it through a sand-filled column. Compared with soil sampling, the catchment area for the spore trap is larger, which should improve detection ability at low inoculum densities. The column is filled with a fixed amount of with autoclaved fine sand mixed with a small percentage of steam-sterilized Yolo loam (to improve water holding capacity). This provides a uniform medium for retaining inoculum that is initially free of other microorganisms. Because this medium is free of *Pythium* spp., the sand can be assayed quantitatively via dilution plating onto PARPH as well as being qualitatively assayed by baiting. Other than filtration and plating of captured rainwater, a labor-intensive method used by the Rizzo lab, the spore trap is the only other monitoring method that is capable of directly quantifying captured *P. ramorum* propagules. However, because captured propagules should survive longer in moist sand than in water buckets, the sand spore trap can be left in place longer after rainfall events before being collected for assay, potentially for many weeks over the rainy season.

To get an initial idea of how the traps would function in the field, we deployed six prototype units at the lower set of plots at the FE location west of Healdsburg on April 22. Three spore traps were placed in the control plot and three were placed in the Agri-fos plot. Traps were left in place for 3 weeks and collected May 13. Rain gauges installed adjacent to two of the traps showed that only 6 cm of canopy throughfall occurred while the traps were in the field. Two traps had drainage problems which we will address in future design modifications. The sand columns were taken to Dave Rizzo's lab for assay. Elizabeth Fitchner baited the soil in the columns using her standard technique and also used dilution plating to assay for *P. ramorum*. All tests were negative, which is not surprising given the general lack of inoculum production this year and the low amount of precipitation that occurred during the test period. We thank Dr. Fitchner for all her work on this phase of the project. We hope to work with the Rizzo lab to conduct some lab testing of the trap so that we will be able to use the traps for next season's inoculum monitoring.

Efficacy

Monitoring disease development on tanoaks within the study plots is our main method for determining whether the Agri-fos® treatment is effective. We assessed the disease status of each tanoak stem in the plots prior to the start of the study and are periodically reassessing the stems to detect evidence of disease. The plots that were established in the winter of 2005/2006 have now been observed for 3.5 years since the start of the experiment. Current disease status for these plots and the change in disease from the start of the study are summarized in Table 1-2.

Compared with the BL plots, the plots at the SF study location were much closer to areas where tanoaks had been killed by *P. ramorum* at the start of the study. It is therefore likely that trees at this location had been exposed to *P. ramorum* inoculum at an earlier date relative to the BL plots. This assumption is supported by the fact that two trees at the SF location had *P. ramorum* canker symptoms before treatments were initiated and died within the first 6 months of the study.

Overall disease levels in all plots are much higher at the SF location than at the BL location (Table 1-2). Plot SF1 (Agri-Fos treated) had the highest incidence of disease overall, but because this plot is also closest to the original *P. ramorum* disease center at this location, it is possible that many of the symptomatic trees in this plot had existing but cryptic cankers at the start of the study. If this is the case, it would support the observation that phosphite application is ineffective at preventing disease progress in tanoaks that are already infected. It is possible that at least some of the infections occurred in early 2006 after the initial phosphite application. If that were the case, it would suggest that the initial application was either not applied early enough to be translocated throughout the tree or that the initial absorbed dose was simply too low to prevent infection.

Table 1-2. Mortality of tanoak stems attributed to *P. ramorum* observed 42 months after initial treatments. Plots were initially treated in December 2005.

Plot	Treatment	Number of live stems at start of study	Number of stems with likely *P. ramorum* canker symptoms	% of stems with likely *P. ramorum* canker	% overall mortality	% mortality attributed to *P. ramorum*
BL3	Agri-Fos+thin	57	1	1.8%	1.8%	0%
BL4	thinned control	57	1	1.8%	7.0%	1.8%
BL5	nonthinned control	56	4	7.1%	5.4%	5.4%
SF1	Agri-Fos+thin	63	17	27%	14.3%	12.7%
SF2	thinned control	61	8	13.1%	1.6%	1.6%
SF6	nonthinned control	72	12*	16. 7%	8.3%	8.3%

*Two of these stems were symptomatic at the start of the study.

Mortality has been observed in all plots since the beginning of the study, but as shown in Table 1-2, some of this mortality is due to causes other than *P. ramorum*. One interesting trend at both SF and BL is that disease levels in the nonthinned control plots are higher than seen in the paired thinned controls. Disease levels are still relatively low overall, so these differences may not hold up over time. Nonetheless, this suggests that inoculum produced on understory trees could have an influence on disease progress. Spore monitoring data would be useful to confirm this hypothesis.

The current disease status of plots established in the winter of 2007 is shown in Table 1-3. Both plot locations are in areas with nearby tanoak mortality due to *P. ramorum*, but disease levels in the plots are currently low. These plots should provide a good test of Agri-fos efficacy because the latent or cryptic infection rate at the start of the study was apparently low. Disease levels in the plots have not changed substantially since the start of the study, presumably due to the low rainfall and correspondingly low inoculum production in early 2007, 2008, and 2009.

Table 1-3. Mortality of tanoak stems attributed to *P. ramorum* observed 30 months after initial treatments. FE1 is paired with FE2, FE3 is paired with FE4. Plots were initially treated in Jan/Feb 2007.

Plot	Treatment	Number of live stems at start of study	% of stems with likely *P. ramorum* canker	Number of stems with likely *P. ramorum* canker symptoms	Increase from start of study
FE1	Agri-Fos+thin	36	0	0	0
FE2	thinned control	30	7%	2	0
FE3	Agri-Fos+thin	34	2.9%	1	1
FE4	thinned control	41	4.9%	2	2
PC2	Agri-Fos+thin	75	4%	3	2
PC1	thinned control	75	2.7%	2	1

Objective 2. Protection of oaks using selective removal of California bay.

We renewed necessary permits and re-established contacts with all cooperators involved in this phase of the study. Starting on 20 June 2009, we returned to all bay removal study locations to:
-evaluated the disease status of study trees;

-remeasure bay-oak clearances and bay cover within 2.5 and 5 m of study trees;
- measure sprout regrowth from cut bay stumps;
- remove or prune additional bay shoots as needed to maintain or improve bay foliage-oak trunk clearances.

We will report on data collected in this round of evaluations in our next progress report.

Presentations:

On 12 May 2009, Ted Swiecki made a presentation on management methods at a SOD training workshop held at Hidden Villa in Los Altos Hills, CA. The talk in included information on Agri-Fos® treatment and bay removal, as well as a question and answer session.

On 21 January 2009, Elizabeth Bernhardt gave a presentation on SOD to the Fairfield Garden Club. The talk covered basic biology of *Phytophthora ramorum* and epidemiology and control of SOD.

Information about this study was included in "Assessing methods to protect susceptible oak and tanoak stands from sudden oak death" by Tedmund J. Swiecki and Elizabeth Bernhardt, presented by Ted Swiecki at the Fourth SOD Science Symposium / COMTF Annual Meeting at Scott's Valley, CA, June 2009.

"Long-term trends in coast live oak and tanoak stands affected by Phytophthora ramorum canker (sudden oak death)" by Tedmund J. Swiecki and Elizabeth Bernhardt, presented by Ted Swiecki at the Fourth SOD Science Symposium / COMTF Annual Meeting at Scott's Valley, CA, June 2009.

We have a synopsis of the study on our website (http://phytosphere.com/publications/SODmanagementstudy.htm) and will be updating this page as current year results are analyzed to share information on project progress with our cooperators and other interested parties. Other websites have cited information from this page.