Management of *Phytophthora ramorum* in tanoak and oak stands
Forest Service Agreement No. 06-JV-11272138-040

Progress report #4 - 6/20/08
This progress report covers the period January-June 2008. 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 Elizabeth Fitchner in the Rizzo lab.
- collected leaf samples for a bioassay to assess phosphite activity in Agri-Fos treated and control tanoak trees. The bioassay was conducted by the Garbelotto lab.

Coast live oak and California black oak bay removal study
- reevaluated disease status of all oaks in the study
- remeasured bay-oak clearances in all bay removal plots and controls
- conducted additional bay pruning or removal as necessary to maintain target clearances
- measured growth of bay sprouts from bases of removed stems

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.

Plots at the SF and BL sites were established in cooperation with the Kashia Band of Pomo Indians of Stewarts Point Rancheria, and most 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	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	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	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	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 and June 2008. Stems in Agri-Fos treated plots were assessed prior to being resprayed. Dead stems and stems that were nearly dead were be 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. Since a number of large-diameter trees in plot SF1 had died (Table 1-2), the total amount of spray solution needed was substantially reduced.

All treated plots were retreated with Agri-Fos in May 2008. Plot SF1 was sprayed using an ATV-mounted sprayer with a 25 gallon (95 L) spray tank. The remaining plots were sprayed using a 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) to maximize the opportunity for phosphite uptake by using a long telescoping spray wand. 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.

When we revisited plots in May 2008, we discovered that a logging skid trail had been constructed through one of the plot sets at the FE site, and a number of the tagged study trees had been bulldozed. According to the landowner, damage occurred during logging operations while he was out of town. We lost only 6 tanoak stems in the Agri-Fos treated plot (FE3), but half of the stems in the paired control plot FE4 (25 of 50) had been destroyed. The portion of the plot that was destroyed had no detectable disease in 2007. As discussed below, the likelihood of many new infections occurring in spring 2008 was low due to lack of rain and low inoculum production. We therefore added 17 new asymptomatic tanoak stems that were adjacent to the remaining FE4 control trees to this plot to replace the destroyed trees. We tagged and collected baseline health data on these additional control trees in June 2008.

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.

Late winter and spring rainfall amounts in 2008 had been looking even bleaker than seen in 2007, so we anticipated that there would not be enough rainfall for the floating bay leaf baiting technique to succeed this year. As it turned out, little or no measurable precipitation fell in the study areas over the intended baiting period in this record drought year.

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. Dr. Fitchner traveled to the Creighton Ridge area with us on 14 May 2008 and collected soil samples from most of the plots at the BL and PC locations and assisted with the spraying operation at those locations. We collected samples from the remaining locations on 12 and 20 May and transferred them to her.

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. We thank Dr. Fitchner for all her work on this phase of the project.

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 2.5 years since the start of the experiment. Current disease status for these plots and the change in disease from 2007 are summarized in Table 1-2. No newly symptomatic trees were observed in the BL plots, but one of the symptomatic trees in the thinned control plot BL4 has died since last year. To date, all of the stems with *P. ramorum* symptoms have been in the control plots at the BL location.

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.

Furthermore, for all of the SF plots, the mean diameter of trees with *P. ramorum* symptoms is significantly greater than the diameter of asymptomatic trees. Furthermore, 5 of the 7 stems killed by *P. ramorum* in the Agri-Fos plot SF1 had stem diameters greater than 38 cm (15 inches). An additional factor contributing to the apparent lack of efficacy in plot SF1 may be that larger-diameter stems did not receive a high enough dose of phosphite to provide adequate levels of disease protection. The leaf analysis data discussed below will help to address this question.

Table 1-2. Mortality of tanoak stems attributed to *P. ramorum* observed 30 months after initial treatments. Plots were initially treated in December 2005.

Plot	Treatment	live stems at start of study	% of stems with likely *P. ramorum* canker	Stems with likely *P. ramorum* canker symptoms	Increase from 5/07	Number of dead stems attributed to *P. ramorum*	Increase from 5/07
BL3	Agri-Fos+thin	57	0	0	0	0	0
BL4	thinned control	57	1.8%	1	0	1	1
BL5	nonthinned control	55	5.5%	3	0	1	0
SF1	Agri-Fos+thin	63	23.8%	15	1	7	4
SF2	thinned control	61	11.5%	7	1	0	0
SF6	nonthinned control	72	15.3%	11	1	5	1

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. ramoru*m. There has been little change in the plots since they were established, presumably due to the fact that disease pressure has been low due to low rainfall the past two springs.

Table 1-3. Mortality of tanoak stems attributed to *P. ramorum* observed 18 months after initial treatments. FE1 is paired with FE2, FE3 is paired with FE4. Plots were initially treated in Jan/Feb 2007.

Plot	Treatment	live stems at start of study	% of stems with likely *P. ramorum* canker	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*	0	0	0
FE4	thinned control	41*	2.5%	1	1
PC2	Agri-Fos+thin	75	4%	3	2
PC1	thinned control	75	1.3%	1	0

* The number of stems in these plots was reduced between 2007 and 2008 evaluations due to unintended tree removal. Number of stems shown for FE3 reflect the number of stems present in 2008 after logging; for FE4 reflect the number of stems after logging and addition of replacement trees to plot.

Leaf sampling for bioassay

We collected leaf samples from tanoaks trees in treated and control plots in early June for a phosphite activity bioassay that was conducted by the Garbelotto lab. Because we were interested in seeing whether the bioassay would show differences in activity between trees that differed greatly in stem diameter, we stratified the trees to be sampled by stem diameter. From each sampled plot, we collected leaves from two of the largest –diameter trees in the plot and from two trees in the lower quartile of stem diameter. Leaves samples were collected from all Agri-Fos treated plots and from the paired control plots. For locations BL and SF, control samples were collected from the thinned control plots. We also collected leaf samples from the largest diameter tanoak that we have treated with Agri-Fos (DBH=153 cm) and an equally large control tree. These trees were part of the phosphite / acorn composition project we conducted for the Kashia Band of Pomo Indians.

Many of the trees in our study plots have very few low leaves. This is especially the case for the larger-diameter trees. In order to collect leaf samples high in the canopy, we constructed a cutting head which we attached to the end of a telescoping 35 ft (10.7 m) fiberglass measuring pole. This allowed us to reach leaves to a height of about 13 m which allowed us to obtain leaf samples from large-diameter trees that could not have been sampled with standard pole pruners.

Leaves were transferred to the Garbelotto lab where they were challenged with *Phytophthora ramorum*. We have not yet received results from these bioassays.

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

This study is based on matched pairs of SOD-susceptible oaks (coast live or California black oak). The trees within the pairs were matched to the degree possible for known factors that influence disease risk, especially the amount of bay in the immediate vicinity of the trunk. One tree of each pair was designated as the control and was not altered in any way. For the other (treated) tree, we removed bay from the zone nearest to the trunk. We tried to achieve a minimum bay foliage-oak clearance of 2.5 m. Where it could be achieved without excessive effort, we increased the minimum clearance up to about 5 m, especially in the direction of the prevailing storm winds (generally south and west of the tree).

Bay foliage-oak clearance was defined as the minimum distance between vertical lines that were even with the edge of bay canopy and the closest surface of the lower oak trunk. We used a green laser attached to an angle gauge to project the vertical line at the edge of bay canopy to the oak trunk and used a laser rangefinder to measure the distance to the oak trunk. Bay foliage-oak trunk clearance was generally achieved by removing small-diameter bay stems close to the oak and/or bay branches from bays located farther from the oak. In some cases, very high bay canopy could not be reached using our pole pruner (above about 8 m) and the bay stems were too large to fell. In such cases, we removed as much of the lower, shaded bay canopy within the target clearance zone as possible. We have previously observed that bay foliar symptoms of *P. ramorum* are generally much more common on this lower foliage than it is on leaves at the top of the canopy.

Locations used in this study are in Sonoma, Napa, and Solano Counties. At the start of the study, *P. ramorum* was present at all of the study locations and was causing symptoms on bay and at least some oaks. The locations included in the study to date are summarized in Table 2-1.

Table 2-1. Number of bay removal study pairs at study locations.

Location	**County**	**Coast live oak pairs**	**California black oak pairs**	**Initial bay removal date**	**Reevaluation date**
Wall Rd.	Napa	7	--	Jan 2007	6/20/2008
Annadel SP	Sonoma	7	6	Feb 2007	6/23/2008
JT/GVR	Napa/Solano	11	--	March 2007	6/16/2008
Jacobs Ranch	Sonoma	5	4	May 2007	6/17/2008
SA	Sonoma	1	8	November 2007	6/13/2008
Total pairs		31	18		

Because localized bay removal is primarily a preventive treatment, we looked for oaks that were free of obvious stem cankers for use as study trees. However, we also included nine trees with small *P. ramorum* cankers in the study to assess whether disease progress could be slowed via bay removal, by reducing the amount of additional inoculum that lands on already-infected trees.

As shown in Table 2-1, additional trees have become symptomatic since the start of the study, increasing the number of symptomatic trees from 9 to 13. New *P. ramorum* canker symptoms were observed in both control and treated trees, and several were confirmed by isolation onto PARP. *P. ramorum* canker incidence did not differ by treatment. As discussed above, it is likely that most or all trees with *P. ramorum* canker symptoms in June 2008 were already infected at the start of the study in 2007, given the poor conditions for inoculum production in the springs of 2007 and 2008, and the low levels of infected bay leaves seen in 2008. Two implications of this are: (1) we were successful at identifying trees that were at high risk of infection, though a bit late to intervene, and (2) canker symptom expression can be delayed for at least two years after a favorable disease period (springs of 2005 and 2006). Data from our long term plots, also indicates that a latent period of two years is not uncommon for coast live oak.

Tree health and bay clearance were re-evaluated in June 2008 as shown in table 2-1. At the same time, we conducted addition bay pruning and removal of small stems, using only manual hand and pole saws, in situations where: (1) bay regrowth had infringed into the clearance zone established with initial pruning or, more commonly, (2) the clearance was suboptimal after the initial round of pruning in 2007 and could be increased with a few additional cuts.

At the June 2008 evaluation, we also measured regrowth of sprouts from removed bay stems and, as needed, trimmed off the sprouts to help maintain adequate bay-oak clearance and to minimize the amount of bay removal needed later. Although the data on sprout regrowth have not yet been analyzed, the largest bay resprouts were about 1 m tall with a basal diameter of about 1 to 1.5 cm, All were easily removed using loppers. None of the bay foliage on these new shoots showed symptoms of *P. ramorum* infection. In addition, bay resprouts at all locations showed varying amounts of browsing. At several sites, browsing of these shoots was so severe that it was unnecessary to remove any sprouts. Browsers showed a clear preference for these young bay shoots, which are relatively succulent and not woody. Deer appeared to be the primary browsers at most locations, but cattle may also have browsed shoots at two locations. In addition, very few of the pruning cuts made to remove bay branches gave rise to epicormic sprouts. From these results, it does not appear that herbicide treatment of bay stumps to prevent resprouting is needed in areas with moderate to high levels of browsing pressure. Even in the absence of strong browsing pressure, bay resprouts are easily removed after a year. We will continue to follow growth of these bay sprouts to determine whether the amount of sprout growth declines over time.

Table 2-1. Changes in *P. ramorum* infection status among trees with and without nearby bay removed from the date of bay removal in 2007 to June 2008. Early=bleeding *P. ramorum* cankers only. Late=bleeding cankers plus signs of beetle infestation and/or fruiting bodies of *Hypoxylon thouarsianum.*

Species	Treatment	Date	*P. ramorum* canker symptom status			
			Asymptomatic	Early	Late	Dead
Coast live oak	Bay removed	2007	28	2	1	0
		2008	25	2	4	0
	Control (no bay removal)	2007	28	3	0	0
		2008	28	1	2	0
California black oak	Bay removed	2007	17	1	1*	0
		2008	17	1	1	0
	Control (no bay removal)	2007	17	1	0	0
		2008	16	1	1	0

* One asymptomatic tree without bay removal was paired with 2 trees which benefited from removal of the same bays. One of the two bay-removal trees had late symptoms of *P. ramorum* infection in 2007.

Presentations:

On 16 April 2008, Ted Swiecki made a presentation on the study, which included information on Agri-Fos® treatment and bay removal, and fielded questions as part of the SOD Treatments panel at the COMTF annual meeting in San Rafael, CA.

We have posted 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.

In addition, information from this page was incorporated into an article published in the January 2008 issue of Landscape Superintendent and Maintenance Professional magazine.