Management of *Phytophthora ramorum* in tanoak and oak stands

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Introduction

The objective of this study was to test methods for managing *Phytophthora ramorum* canker in tanoak and oak stands. Because disease epidemiology differs between different canker hosts, we tested different control strategies in tanoaks and susceptible oaks. The long term plots we have established for this project are located in Napa, Solano, and Sonoma counties.

For tanoak, we tested potassium phosphite (Agri-fos®) applied as a bark spray to protect trees from *P. ramorum.* Because *P. ramorum* reproduces on tanoak, fungicide application is one of the few strategies that has the potential to protect tanoak stands. Although widely used, preventative application of phosphite to protect trees from SOD is supported by very little field data. This study tests phosphite efficacy in a variety of sites under conditions that are realistic from an implementation standpoint, but are controlled and optimized to provide a reliable test. The design of Agri-Fos® 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.

For coast live oak and California black oak, we are testing whether localized removal of California bay will reduce the risk of *P. ramorum* infection to acceptably low levels. California bay is the primary source of *P. ramorum* spores that infect susceptible oaks in forests lacking tanoak. The risk of *P. ramorum* infection and the severity of disease in susceptible oaks increases as the amount of bay canopy close to an oak increases and as the minimum bay-oak clearance decreases (Swiecki and Bernhardt 2007, 2008). Oaks with bay foliage directly over or within 1.5 m of the trunk have the highest risk of infection and that disease risk increases as the total amount of bay cover within 2.5 to 5 m of the oak trunk increases. The current study examines whether relatively minor amounts of bay removal in the zone closest to the oak trunk can provide a significant reduction in SOD risk.

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

Methods

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

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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 of the activities associated with those locations was previously conducted under a separate contract with the Kashia, with funding provided by USDA-FS State and Private Forestry. That contract has ended, so activities associated with those plots are covered under this project.

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 May 2010	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 June 2010	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 May 2010	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 April 2010	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 individually evaluated for disease status and condition. The number of stems in each plot is shown in Table 1-2. We assessed the disease status of all stems in sprayed plots just prior to the annual Agri-fos® reapplication. Dead stems and stems that were nearly dead were omitted from the list of stems to be sprayed. Prior to treating the BL Agri-fos plot in 2010, we removed one tree in the plot that had a largely horizontal trunk with its tip touching the ground. This tree has been problematic for several years because most of the canopy was within 2 m of the ground surface. As a result, most of the foliage had been burned by overspray from adjacent treated trees. We treated an additional asymptomatic tanoak on the edge of the plot in June 2010 as a replacement.

Agri-Fos was applied using a 4 gal (15 L) ShurFlo Propack electric backpack sprayer mounted on a modified mountain bike. For all applications, we banded the spray high on the stem (3 to 6 m height) using a long telescoping spray wand to maximize phosphite uptake. To minimize the variation in the phosphite dose applied to trees of different sizes, we calculated a specific diameter-related spray volume for each stem. Methods used to calculate the spray volume for each stem were as described in our December 2007 progress report. Application equipment was calibrated to apply a known spray volume per unit time, and spray applications for each tree were timed to apply the desired volume. Total applied spray volumes were typically within 1-2% of the calculated target amounts.

Results and discussion

Monitoring disease development on tanoaks within the study plots was our primary method for determining the efficacy of the Agri-fos® treatment. We assessed the disease status of each tanoak stem in the plots prior to the start of the study and have periodically reassessed the stems to detect evidence of disease. Through 2010, the plots established in the winter of 2005/2006 had been observed for 4.5 years. The disease status for these plots in May/June 2010 and the change in disease status from the start of the study are summarized in Table 1-2.

The plots at the SF study location were relatively close (within 50-100 m) to California bays and tanoaks that were infected by *P. ramorum* at the start of the study. These plots were therefore likely to have been exposed to *P. ramorum* inoculum prior to the start of the study and continued to be exposed to high levels of inoculum over the course of the study. 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. In comparison, at the start of the study, the nearest trees with SOD symptoms in the vicinity of the BL plots were more than 200 m away in a drainage at an elevation well below the plot area. It appears that the BL plots were exposed to little or no *P. ramorum* inoculum prior to the start of the study.

By 2010, overall disease levels in all plots were 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 this plot is also closest to the original *P. ramorum* disease center at this location (within about 50 m). It is possible that many of the symptomatic trees in SF1 had existing but cryptic cankers at the start of the study. If this was the case, we would conclude that phosphite application was ineffective at preventing disease progress in tanoaks that were 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, we would conclude 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.

At the BL location, SOD incidence in all plots was still very low in 2010. Although disease levels in control plots are slightly higher than in the Agri-fos®-treated plot, overall disease levels in the controls are still too low to show a significant treatment effect.

Table 1-2. Mortality of tanoak stems attributed to *P. ramorum* observed about 4.5 years (May/June 2010) 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 symptoms	% overall mortality	% mortality attributed to *P. ramorum*
BL3	Agri-Fos®+thin	57	1	1.8%	1.8%	0%
BL4	thinned control	57	6	10.5%	8.8%	3.5%
BL5	nonthinned control	56	6	10.3%	8.9%	7.1%
SF1	Agri-Fos®+thin	63	19	30%	17.5%	15.9%
SF2	thinned control	61	8	13.1%	3.3%	3.3%
SF6	nonthinned control	72	12*	16. 7%	9.7%	9.7%

*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 trend at both SF and BL is that mortality attributed to *P. ramorum* is greater in the nonthinned control plots than in the paired thinned controls. However, differences in overall disease levels between these treatments has become less pronounced over time. This suggests that removal of understory tanoaks may delay but not necessarily stop SOD development in newly-infested stands. If this is the case, understory tanoak removal may help improve efficacy in chemical control treatments and could also have value in "slow the spread" efforts. Further research in this area is warranted to better characterize the role of understory tanoaks on overall inoculum production within stands.

Table 1-3 summarizes the 2010 disease status of plots established in 2007. Both plot locations are in areas with nearby tanoak mortality due to *P. ramorum*, but disease levels in the plots were still low in 2010. These plots should provide a good test of Agri-fos® efficacy because the latent or cryptic infection rate at the start of the study appears to have been 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. As described in section 3 below, even though spring rainfall was relatively high in 2010, we were unable to detect spore production in either the treated or untreated plots.

Table 1-3. Mortality of tanoak stems attributed to *P. ramorum* observed about 3.5 years (April/May 2010) 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	Number of stems with likely *P. ramorum* canker symptoms	% overall mortality	% of stems with likely *P. ramorum* canker
FE1	Agri-Fos+thin	36	0	5.6%	0
FE2	thinned control	30	2	3.4%	7%
FE3	Agri-Fos+thin	34	1	2.9%	2.9%
FE4	thinned control	41	2	2.4%	4.9%
PC2	Agri-Fos+thin	75	3	2.6%	4%
PC1	thinned control	75	2	1.3%	2.7%

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

Methods

In this portion of the project, we used a matched pairs design to study the effect of localized bay removal around SOD-susceptible oaks (coast live or California black oak). The oaks 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 trunk 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). If present, poison oak climbing in the tree canopy was killed by cutting stems at ground level.

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 nearest to the oak trunk and used a laser rangefinder to measure the horizontal distance from this line to the oak trunk. Bay foliage-oak trunk clearance was generally achieved by removing small-diameter bays 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.

In addition to measuring the minimum bay foliage-oak clearance, we also assessed the bay neighborhood around each oak. We scored bay canopy cover within 2.5 m of the oak

trunk and within 5 m of the oak trunk using a 0-4 scale (0=no bay canopy, 1=1-25% cover, 2=26-50% cover, 3=51-75% cover, 4=more than 75% cover). We also recorded whether bay was present within 5-10 m and 10-20 m from the oak trunk. The presence of bay foliar symptoms was also noted for the zones from 0-2.5 m and from 2.5-5 m from the oak trunk.

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
Wall Rd.	Napa	7	--	Jan 2007
Annadel SP	Sonoma	7	6	Feb 2007
JT/GVR	Napa/Solano	11	--	March 2007
Jacobs Ranch	Sonoma	5	4	May 2007
SA	Sonoma	1	8	November 2007
Total pairs		31	18	

About a third of the trees in the study have more than one trunk. Because most *P. ramorum* cankers are located well above soil level, SOD symptoms typically progress independently in separate trunks. If different stems show different disease status (e.g., asymptomatic and dead) reporting disease status for the whole tree becomes problematic. Hence, SOD disease status variables and bay clearance were recorded separately for each trunk.

Because localized bay removal is primarily a preventive treatment, we looked for oaks that were free of obvious stem cankers to use as study trees. However, we also included 9 stems 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.

Results and discussion

The number of study tree stems with SOD symptoms has doubled to 18 since the start of the study. However, no newly symptomatic trees were seen in 2010. In a few stems, observed symptoms have gone into remission, so the number of stems with active symptoms has varied from year to year.

As discussed in previous progress reports, most or possibly all of the stems that have developed *P. ramorum* canker symptoms through July 2010 were likely to have been infected before the start of the study in 2007. Data from our long term plots indicates that the latent period from infection to symptom development is commonly two to three years

for coast live oak. Weather conditions were favorable for infection in 2005 and 2006, but conditions in spring 2007, 2008, and 2009 were unfavorable for *P. ramorum* sporulation.

We observed low numbers of *P. ramorum*-infected bay leaves in the plot areas in both 2008 and 2009 and greater numbers in 2010, which had weather conditions that were relatively favorable for *P. ramorum* sporulation. As described in section 3 below, *P. ramorum* was baited from a spore trap set up under a bay near one of the control trees at Annadel SP in spring 2010.

We evaluated the disease status of study trees annually. As of the July 2010 evaluation, *P. ramorum* canker incidence did not differ by treatment (Table 2-2). This result is not surprising, given the lack of likely infection periods from the start of the study through 2009. Infections that may have been initiated in spring 2010 would not be likely to show symptoms by July 2010. Summer 2011 evaluations would provide the first chance to see if any study trees were infected in 2010 and whether the bay removal treatment affected disease outcomes.

We remeasured bay foliage-oak trunk clearance distances and bay cover in July 2010. Bay-oak clearances have not changed for controls because their clearances were mostly zero at the study start. However, bay cover ratings in the zone closest to the control oaks did increase slightly between 2008 and 2010. The bay cover score for the 0-2.5 m distance zone increased by 0.22, which was significant at $p<0.001$ using Wilcoxon signed rank test).

In contrast, most oaks with bay removal have shown some loss of clearance each year. From 2008 to 2009, 34 treated oaks (69%) had lost clearance. The mean loss in clearance was 0.33 m. Results were similar for the interval from 2009 to 2010 (63% lost clearance, mean clearance loss 0.26 m). For almost all trees, less than 1 m of clearance was lost per year. For oaks with the bay removal treatment, bay cover ratings in the 0-2.5 m zone around have not changed since 2008.

To help maintain acceptable the clearance zones, we have conducted additional bay pruning and removal of small stems as needed. All follow-up pruning has been performed using only manual hand and pole saws. In 2010, we removed bay stems or branches from around 15 of the 49 oaks with the bay removal treatment. Only one of these trees had a minimum clearance less than the target 2.5 m minimum. For the other trees, pruning was done to either further improve clearance or minimize the need for larger pruning cuts in subsequent years. For some other trees that were at or near the minimum target clearance of 2.5 m, we removed additional low understory bay canopy, even if this removal did not increase overall clearance.

Table 2-2. *P. ramorum* infection status among oak trunks with and without nearby bay removed in 2007. No trees were dead in 2007. Early=bleeding *P. ramorum* cankers only. Late=bleeding cankers plus signs of beetle infestation and/or fruiting bodies of *Annulohypoxylon thouarsianum.*

Species	Bay removed	Asymptomatic 2007	Asymptomatic 2010	Early Pr 2010	Late Pr 2010	Dead Pr 2010
QA	no	78%	80%	10%	7%	2%
QA	yes	81%	76%	3%	16%	5%
QK	no	96%	87%	4%	4%	4%
QK	yes	92%	92%	4%	0%	4%

Bay sprout growth. At the July 2010 evaluation, we measured regrowth of sprouts from removed bay stems. To date, we have not observed symptoms of *P. ramorum* infection on the foliage of bay stump sprouts.

Bay resprouts at all locations showed varying amounts of browsing. Annadel SP has consistently shown the most severe browsing of bay resprouts, whereas browsing has been lightest at SA. Native vertebrates, primarily deer, are present at all locations and are the only browsers at these two locations. JT/GVR and Jacobs Ranch are grazed seasonally by cattle, and horse grazing occurs over much of the year at Wall Road. Livestock browsing may contribute to the suppression of sprout regrowth at these three locations (figure 2-1). Browsers showed a clear preference for the young bay shoots that arise from cut stumps. These shoots are relatively succulent and not very woody initially.

Where browsing has been insufficient to suppress shoot regrowth, we have used an axe or hand saw to cut off long sprouts after shoot measurements are made. In 2009, we trimmed sprouts on 28% of the 84 monitored bay stumps. In 2010, we trimmed sprouts on 33% of the monitored stumps. Sixteen of the stumps (19% of all stumps) had sprouts trimmed in both years. Due to suppression by browsing and mechanical shoot removal, mean bay sprout heights in 2009 and 2010 were significantly lower than 2008 mean heights ($p<0.05$, Tukey-Kramer HSD; figure 2-2). Suppression of sprout regrowth appears to have reduced the vigor of the stumps to some degree, but none of the monitored stumps had died by July 2010.

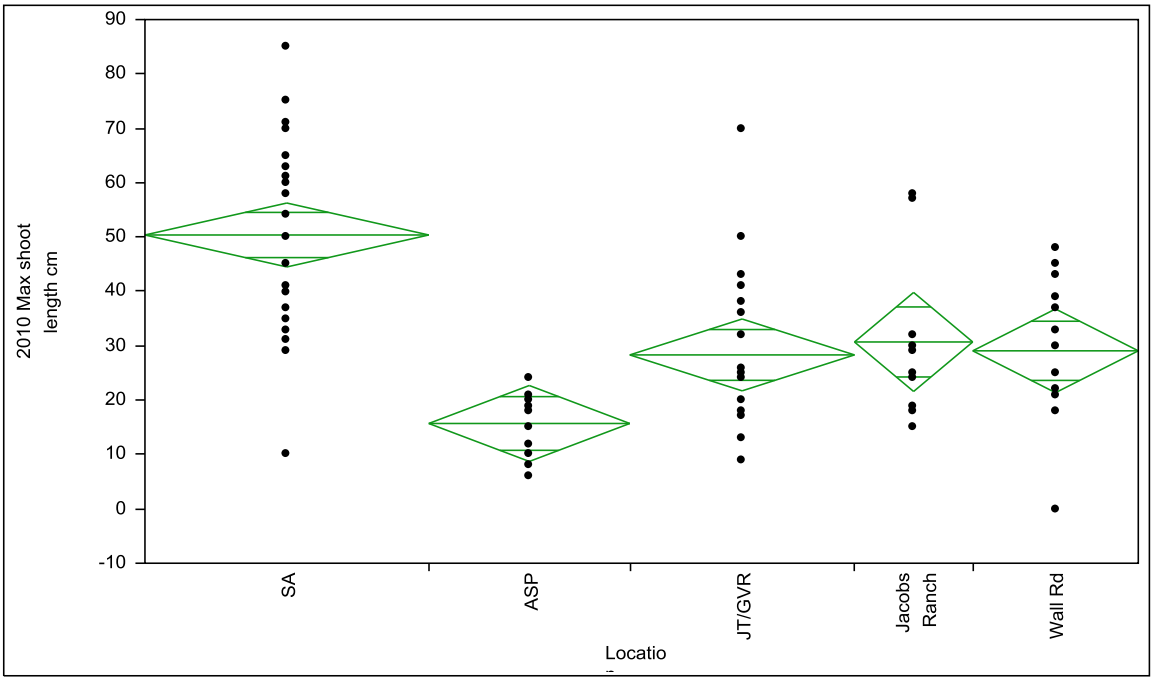


Figure 2-1. Maximum height (cm) in July 2010 of bay sprouts from stumps of trees cut in 2007. Location means are shown by the horizontal line in center of each diamond.

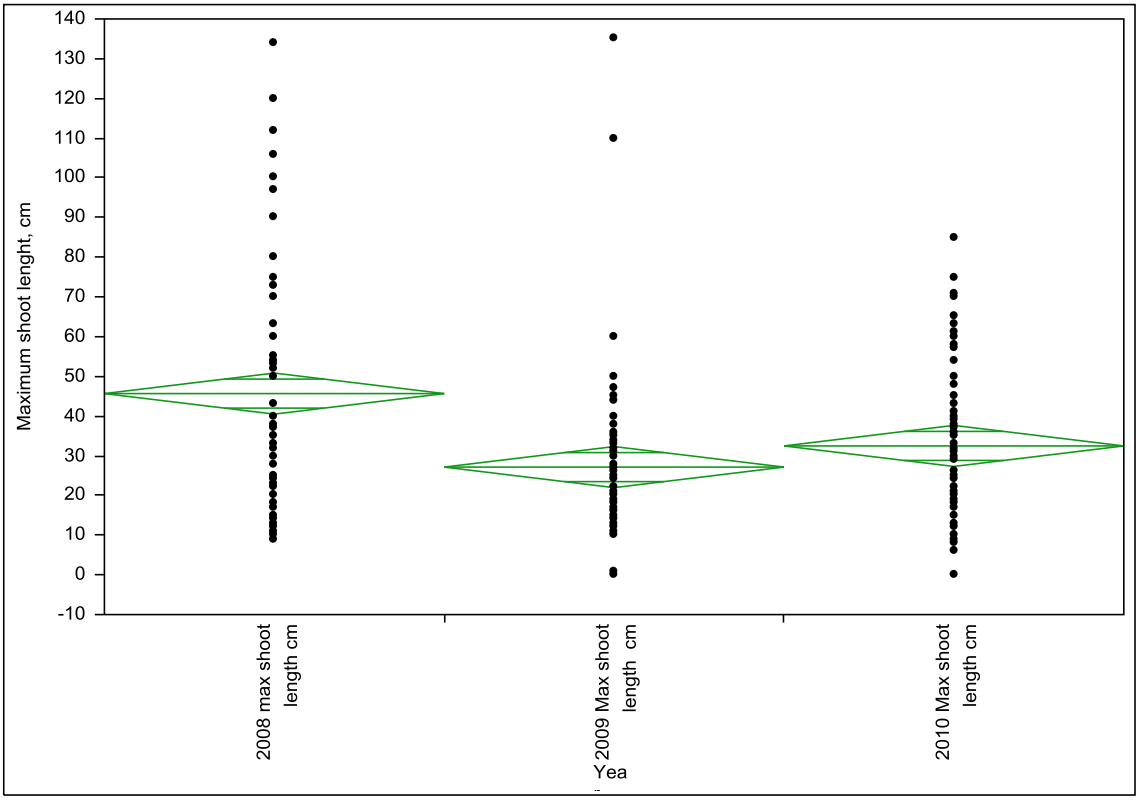


Figure 2-2. Maximum height (cm) of bay resprouts in June 2008, June 2009, and July 2010 from stumps cut in 2007. Means are shown by the horizontal line in each diamond.

3. Development and testing of spore traps for use in tanoak and oak SOD management studies

Over the course of this study, we have used several methods to monitor inoculum production in disease management plots. The initial methods used were not entirely satisfactory. The bay leaf baiting method (floating bay leaves in open buckets of water for several weeks, see progress report 3, December 2007) was relatively labor intensive, and was not particularly sensitive. It is also susceptible to artifacts associated with inoculum cycling within the water or degradation of the bay leaves. Soil sampling (collecting and baiting soil samples collected within plots, see progress report 4, June 2008) was somewhat less labor intensive but also failed to detect low inoculum levels. Both methods are strictly qualitative, providing only presence/absence data for each sample. Only the rainfall capture methods used by the Rizzo lab provide quantitative information about inoculum levels, but the methodology is very labor intensive. Our goal in testing a sand-based trap was to find a method for measuring inoculum production within plots that:
- was not overly labor intensive,
- was relatively insensitive to sources of interference and variation that affect the soil and bay bait methods, and
-has the potential to provide quantitative data on inoculum levels in treated vs. untreated plots.

The current version of the sand spore trap is illustrated in figure 3-1.

Spore trap lab tests

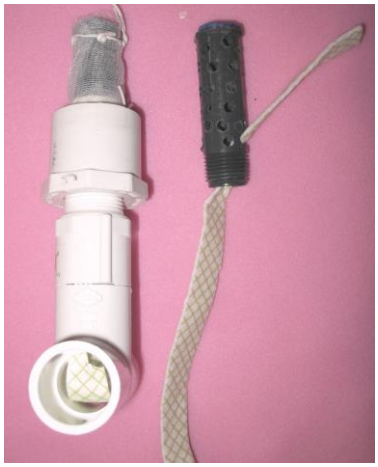
Starting at the end of August 2009, we began controlled lab-based tests to check the performance of our prototype traps, which we had deployed for a few weeks at one field location in 2009 as noted in progress report 6 (June 2009). The lab tests were designed to help optimize retention and survival of *P. ramorum* propagules.

Lab test 1. In the first test, we diluted *P. ramorum* zoospore suspensions to concentrations that had been documented in captured rainwater (about 100 propagules/ml). We applied 3 L of suspension to our spore traps (about 3.37×10^5 zoospores) and captured the effluent that passed though the spore trap columns. We filtered two 100 ml aliquots of the effluent from four columns through Millipore filters and plated the filters onto PARPH to estimate the number of viable *P. ramorum* propagules that passed through the columns. The results of that assay indicated that approximately 99.93% of the zoospores from the applied zoospore suspensions were retained in the spore trap columns. This indicated that the spore traps were retaining a sufficiently high percentage of the inoculum present in the suspension.

We subsequently maintained spore trap columns in the lab for up to 22 days to assess the survival of *P. ramorum* propagules over time. Soil samples from two replicate columns were collected 8, 15, and 22 days after the zoospore suspensions were added. Soil was assayed directly by plating and the remaining soil in each column was baited with rhododendron leaf disks .

Assembled column with one screen removed and wick pulled out

Complete spore trap deployed in field



Filter fabric is wrapped around the drain stem and a wick extends from the bottom end of the drain stem to the outside of the column.

Upper end of filled column showing sand/loam mixture and baffle in intake.

Figure 3-1. Spore trap used in 2010. The completed trap assembly (top left) consists of a tray with a sand/loam filled chamber that retains captured inoculum. Details of the column assembly are shown. A fine mesh fabric is used to retain sand in the column but permit water drainage. A synthetic wick attached to the drain helps the sand/loam mixture drain to near field capacity.

Soil plating was conducted by spreading a known volume of soil from each column onto each of five PARPH plates. After plates were incubated for 3 days and propagules had a chance to germinate and start growing into the agar, plates were rinsed to remove sand from the surface of the plate. Plates were incubated for an additional 4 to 11 days and the number of *P. ramorum* colonies on each plate was counted under magnification.

For baiting, rhododendron leaf disks were placed in containers with flooded soil for 3 or 7 days. At the end of the baiting period, disks were rinsed and plated onto PARPH.

P. ramorum propagules were detected by both soil plating and baiting at each of the sample dates. However, the number of propagules per ml of soil detected by direct plating was relatively low after 8 days and decreased by about an order of magnitude each succeeding week. This rapid loss in survival was greater than expected, given the relatively long persistence of *P. ramorum* in soil. Although a number of factors could contribute to the rapid loss of viability, one important factor was that columns remained saturated for the entire incubation period rather than drying down to field capacity as would typically occur in a forest situation. This may lead to lower oxygen levels and/or greater activity of microbial antagonists.

Lab test 2. We conducted further lab studies in the Rizzo lab in January 2010 to test the performance of a modified sand spore traps design. The column design was altered by adding a wicking material to the bottom of the column to improve drainage of the columns.

The second lab study was initated on 25 January 2010 and generally followed the methods as described for the first test above, except that additional assays were conducted at time 0,. i.e., the time that that zoospores were originally added to the column. We prepared a stock *P. ramorum* zoospore suspension in the laboratory and determined its concentration with a hemocytometer. We diluted the zoospore suspension to 180 propagules/ml using deionized water. We poured the 3 L of the diluted suspension (about 5.42×10^5 total zoospores) into a plastic tray connected to a PVC column filled with 160 g (air dry, about 100 ml) of an autoclaved mixture of fine sand and Yolo fine sandy loam (5:1 v/v). The effluent that passed through the column was collected for assay as described below.

After the suspension had drained through the column, the column was disconnected from the tray and placed upright in a plastic beaker for incubation in a growth chamber (20 C day / 10 C night), except for time 0 samples which were immediately sampled as described below. Eight replicate columns were used to filter zoospore suspensions. Two columns were sampled at time 0 on 25 January 2010, and two columns each were sampled 1, 2, and 3 weeks after 25 January 2010. We also made four control columns by passing 3 L of deionized water (with no added zoospores) through each column. One control column was sampled at time 0, and weeks 1, 2, and 3,

The effluent that passed though the spore trap columns was also assayed to determine how many *P. ramorum* propagules passed though the column. We filtered a 100 ml aliquot of the effluent from each of the eight *P. ramorum* columns through Millipore

filters and plated the filters onto PARPH. On average 23 colonies were detected in the effluent of the columns. This suggested that only about 676 propagules were detected in the effluent, only about 0.1% of the 5.42×10^5 total zoospores added to each column.

While this implied a relatively high efficiency of propagule capture, the situation is complicated by the relative efficiency of detecting viable propagules using various assay methods. To test our detection efficency on PARPH, we placed 0.5 ml aliquots of the diluted (180 propagules/ml) zoospore suspension on PARPH media, and counted the resulting colonies. Only 21% of the zoospores present (based on hemocytometer counts) produced colonies.

We also diluted our stock zoospore suspension to create 100 ml of a zoospore suspension at 180 zoospores per ml. This suspension was filtered through a Millipore filter and plated onto PARPH to estimate the efficiency of recovery after filtering. Based on colony formation on PARPH, only 0.25% of the zoospores in the suspension gave rise to colonies after filtering through the Millipore filter. This implies that either sampling error in pulling aliquots from the zoospore suspension resulted in a different zoospore concentration than intended or that the Millipore filter plating method greatly reduced survival of the zoospores. Taking recovery efficiency into account, these results imply that the actual number of zoospores in the effluent could have been as much as 3.54×10^5 zoospores, or 51% of the zoospores added to the column.

At 0, 1, 2 and 3 weeks after the start of the study, we sampled two columns that had received *P. ramorum* suspensions and one control column. Sand mixture was removed from the column and thoroughly mixed. A small subsample was taken to measure gravimetric moisture content. Known volumes of sand from each column were also placed onto each of five PARPH plates and spread out evenly across the surface of the plate. The remaining sand from the column was flooded with water and 10 rhododendron leaf disk baits were floated on the water surface.

Sand plates were incubated for three days to allow *P. ramorum* propagules to germinate and start growing into the agar. Plates were then rinsed with deionized water to remove sand from the surface of the plate. Plates were incubated for an additional 4 to 11 days and observed periodically to mark and count the number of *P. ramorum* colonies on each plate under magnification. Half of the rhododendron leaf disks used for baiting were removed after 3 days, rinsed, and plated onto PARPH. The remaining disks were removed and plated after 7 days. Baiting efficiency was equal for the two different incubation periods.

We detected *P. ramorum* propagules by both sand plating and baiting at each of the sample dates. The number of colonies per column detected by soil plating at time zero averaged 1.69×10^4. This number represents only about 3% of the propagules that were added to the columns (based on hemocytometer counts). This suggests that the plating efficiency of the sand is well below the 21% efficiency seen in plating zoospore suspension directly onto PARPH. We have not conducted additional tests to determine the detection efficiency of the sand plating operation.

The number of propagules per ml of soil detected by direct plating decreased by about a factor of 4 from week 0 to week 1, but remained relatively constant from week 1 through week 3 (Figure 3-2). This survival was much better than observed in our original column design (Lab test 1) and may be in part due to design changes that reduced water logging of the sand in the columns. The relatively constant survival seen from week 1-3 indicates that the time that a column is collected and assayed after the last rain event is not likely to be overly time sensitive. The propagules used in this lab study were primarily zoospores, although some sporangia were observed in the suspension. It is possible that more of the propagules that are dispersed in the field are actually sporangia, which could result in better initial survival and survival over time than seen in this study.

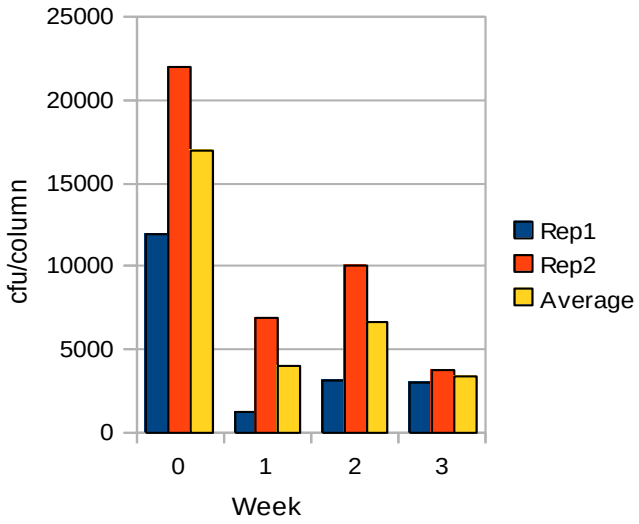


Figure 3-2. Average number of *P. ramorum* propagules detected per spore trap column via soil plating at different dates after input of zoospore suspensions. Rep 1 and rep 2 represent the two different columns harvested at each date. Yellow bars represent the average of both columns.

All baits incubated in soil from columns with added zoospores were positive for *P. ramorum* at each sample date and baits added to soil from control columns were negative. While these results show that soil baiting is a relatively sensitive means for detecting *P. ramorum*, it also emphasizes the non-quantitative nature of most baiting assays: baiting efficiency did not vary with propagule concentration.

Inoculum monitoring in plots

Although lab tests provided information about how the spore traps function under a specific set of controlled conditions, field conditions are much more variable. Field conditions also differ from lab tests with respect to types and numbers of propagules present, precipitation rates, the timing of inoculum shower events, and many other variables. Consequently, the only way to determine how the traps function under field conditions is to test them in the field. In spring 2010, we tested the traps at two of our tanoak / Agri-fos plots (FE and PC locations). We also tested the traps at Annadel SP (discussed in section 2) and at Monte Bello OSP in San Mateo County in plots

established under a separate project funded by FS-State and Private Forestry (08 DG 11052021-144) and the Midpeninsula Regional Open Space district.

Tanoak locations

We used the sand spore traps described above to monitor *P. ramorum* spore production in the plots at the FE and PC study locations (Table 1-3). Three traps were set up in both Agri-Fos® treated and control plots at both locations. At the FE location, traps were left in place for about a month prior to the Agri-Fos® reapplicaton, from 26 March to 22 April 2010. During this interval, 11.3 cm of rain was measured in a rain collector mounted adjacent to one of the spore traps. At the PC location, traps were in place for just over a week, from 24 May to 1 June 2010. We measured 2.6 cm of rainfall in rain collectors mounted adjacent to traps at this location. Although the total amount of precipitation was low, it fell over several days in what was a relatively wet late spring period that should have been favorable for inoculum production. Nonetheless, we did not detect any *P. ramorum* in any of these spore traps either by baiting or plating of the sand from the columns. As discussed in previous progress reports (June 2007, 2008, and 2009), we have not yet detected *P. ramorum* inoculum in these plots using other techniques (bay leaf buckets and baited soil samples). In conjunction with the low amounts of disease in the plots (Table 1-3), these result suggest that the plots have not yet been infested with *P. ramorum* to a significant degree.

Oak locations

We used the sand spore traps to assess inoculum deposition near study trees at Annadel State Park over two time intervals (Table 3-1). Traps were set up adjacent to trunks of four trees from which bay had been removed and four control trees which had not had any bay removal. All control traps were located under bay canopy, whereas the traps near the bay removal trees were 3 to 5.8 m from bay canopy.

The first trapping interval at Annadel was the same used for the FE tanoak plots. All traps at the two tanoak locations drained properly, and very little detritus was found in the traps, which were all covered with 1 cm mesh plastic screening material. At Annadel, however, oak worm feeding activity in the oak canopy was intense during the first trapping interval (3/26-4/22/10). As a result, most of the spore traps became clogged with oak worms and oak worm frass that fell into the traps. Only two of the eight were properly drained when collected on 22 April 2010. The remaining traps had varying amounts of standing water and the columns were saturated. No *P. ramorum* inoculum was detected in this initial round of trapping. Although saturation of the traps may have affected detection efficiency, it should be noted that bay foliar symptoms were not observed in the vicinity of the traps when they were deployed (3/26/10) or picked up (4/22/10).

We redeployed 4 fresh traps on 22 April 2010 around two pairs of trees that were in the portion of the study area that had greater bay cover and more SOD overall. When this second set of traps was removed on 3 June 2010, bay leaves around one of the control trees (974) had developed extensive foliar necrosis typical of *P. ramorum* infection and we isolated *P. ramorum* from the necrotic areas of sampled leaves. The spore trap under

this tree was at least partially clogged and the trap contained some standing water. The other three traps had drained. We obtained a positive detection of *P. ramorum* from the baited sand from the column from tree 974, but did not detect inoculum in the soil plating assay.

Table 3-1. Results of spore trapping near oaks at Annadel State Park with and without bay removal. *P. ramorum* detection is through baiting of the sand in the spore trap with rhododendron leaf disks. Tree numbers shown in bold had columns that were drained at the time of sampling. Columns by other trees had clogged to varying degrees and had standing water in the tray.

Tree numbers	Treatment	Time period	Rainfall (cm)	Sand baiting	Sand plating
265, 269, **972**,977	Bay removal	3/26-4/22/10	9.4	Negative	Negative
268, **272**, 971, 974	Control	3/26-4/22/10	9.4	Negative	Negative
972, 977	Bay removal	4/22-6/3	5.0	Negative	Negative
971, 974	Control	4/22-6/3	5.0	947 Positive	Negative

Spore traps were also set up in a Shreve oak stand in the Monte Bello Open Space Preserve between 18 March and 14 April 2010 (interval, 22.7 cm rain) and 14 April to 13 May 2010 (2.8 cm rain). We detected *P. ramorum* by baiting only in two traps at this location in the first interval (one under bay, the other 30 m from bay canopy) and in one trap during the second interval (the previous positive trap location under bay).

These results suggest that the sand traps have the potential to be used for inoculum monitoring in SOD management plots, although further work to improve detection efficiency is warranted, especially in the quantitative sand plating assay. Reliable, quantitative, but relatively inexpensive spore traps such as this have the potential to provide valuable information for both SOD management and detection/monitoring

Presentations

A synopsis of the study is posted on our website (http://phytosphere.com/publications/SODmanagementstudy.htm) and has been updated to include information from the study through 2010. The webpage provides a way to communicate research results with our cooperators and other interested parties. The information on this page has been cited on other websites.

Citations

Swiecki, T. J.; Bernhardt, E. A. 2007. Influence of local California bay distribution on the risk of *Phytophthora ramorum* canker (sudden oak death) in coast live oak. Final Report. Prepared for Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture Berkeley, CA. 30 p. http://phytosphere.com/publications/influence_bay_dist_SOD.htm

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