Sudden Oak Death Management and Monitoring in the Bay Area
Forest Service Agreement No. 11-DG-11052021-111

Progress report Aug - Dec 2011

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Summary: Agri-fos applications were completed at sprayed sites, and additional bay were removed from some plots at Los Trancos and Russian Ridge. Disease evaluations were completed at all sites. Some data entry and initial summaries have been completed. Very few newly symptomatic trees were observed across all locations.

Project objectives

Objectives for the project are listed below. This contract continues management and monitoring projects that began in 2008 under contract 08-DG-11052021-144 and continued under contract. 10-DG-11052021-214. This project is jointly funded by the Midpeninsula Regional Open Space District (MROSD) for management projects on District lands. Funding for activities on San Francisco Public Utilities Commission (SFPUC) lands are provided entirely by SFPUC, and serve as a source of matching funding for this project.

1. Continue management projects designed to protect vulnerable but currently non-diseased stands of tanoak by treating large forest patches with Agri-fos® via bark spray application in plots located at:

    A*. SFPUC lands in the Peninsula Watershed near Crystal Spring Reservoir (Skyline Drive)

    B. MROSD El Corte de Madera Open Space Preserve.

2. Continue treatments and monitor effectiveness of the combined use of localized bay removal and Agri-fos® treatments for protecting large, high value oaks at:

    A. MROSD Rancho San Antonio Open Space Preserve (coast live oak).

    B. MROSD Los Trancos Open Space Preserve (canyon live oak).

3. Monitor the effectiveness of area-wide bay removal to protect vulnerable stands of oaks at:

    A. MROSD Rancho San Antonio Open Space Preserve (coast live oaks)

    B. MROSD Monte Bello Open Space Preserve (Shreve oaks)

    C*. SFPUC Pulgas Water Temple vicinity, Peninsula Watershed (coast live oaks)

    D. MROSD Russian Ridge Open Space Preserve (canyon live oak)

4. Monitor the effectiveness of cut stump herbicide treatments for suppressing bay resprouting in Rancho San Antonio Open Space Preserve and Monte Bello OSP.

5. Collect data on long-term SOD monitoring plots established in 2000 (Marin, Sonoma, Napa Co.) to maintain data continuity on disease incidence, symptom progress, tree mortality, and tree failure.

6. Monitor the disease development in both inoculated and naturally-infected canyon live oak to determine how to diagnose SOD on this species

*Full funding for this project phase is provided by SFPUC; no FS funds are use for these project locations.

Summary of project activities

A summary of management projects undertaken to date is shown in Table 1.

Table 1. SOD management studies initiated on MROSD lands from 2008 through 2010.

Open Space Preserve	Locality	Host species present[1]	Treatment(s) and dates applied	Treated sample size	Control sample size
El Corte de Madera (ECDM)	near gate CM06	**tanoak**, coast live oak, Shreve oak, canyon live oak	Agri-Fos stem spray application with removal of small understory tanoak: Jan 2009 May 2009 Nov 2009 Nov 2010 Nov 2011	158 stems	164 stems
Monte Bello	Skid Road trail gate (MB06)	**shreve oak**, canyon live oak	areawide bay removal (includes hack and squirt bay treatments): Dec 2008 /Mar 2009 bay removal, stump treatment, hack and squirt July 2009, May 2010, Dec 2011 bay hack/squirt	97 stems	86 stems
Rancho San Antonio (RSA)	permit lot area	**coast live oak**	Localized bay removal (Nov 2008) and Agri-Fos injection: Nov 2008, Jan 2011.	9 stems[2]	61 stems
			Localized bay removal (Nov 2008) and Agri-Fos stem spray application: Jan 2009, May 2009, Nov 2009, Nov 2010, Nov 2011	14 stems*	
			Areawide bay removal only: Nov 2008	42 stems	
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 Agri-Fos spray application: Nov 2009, April 2010, Nov 2010	16 stems	29 stems[3]
			Localized bay removal only: Dec 2009, April 2010, summer 2011	9 stems	
Russian Ridge	Near Ancient Oaks Trail	**canyon live oak**	Localized bay removal: Dec 2009, Sep 2010, summer 2011	36	34[4]
Skyline	Rattlesnake Point area	**canyon live oak**	Inoculation of canyon live oak to complete Koch's postulates, observe symptom progresssion, assess isolation efficiency	18 canyon live oak, 2 Shreve oak	

[1] Bold font face= primary species
[2] One sprayed tree was removed in 11/09. One injected stem of a multistemmed oak failed in 2009., and the three remaining stems were switched to spray application in 2010. As a result, the number of injected stems changed from 13 to 9 and sprayed stems from 11 to 14.
[3]Two stems moved to 'Localized bay removal only' category after additional bay clearance in summer 2011.
[4]Two stems moved to treated category after additional bay clearance in summer 2011.

1. Management of SOD in tanoak with Agri-fos bark spray applications,

A. Crystal Springs Reservoir at Skyline Drive

Tanoak plots near Crystal Springs Reservoir were evaluated for disease in September and October 2011. *Phytophthora ramorum* was first seen in control plots in this plot in the spring of 2011. Widespread foliage and twig blighting is now present on understory tanoak throughout the treated and control plots. We have not yet seen any SOD-caused bole cankers, but additional tree mortality due to other factors has occurred. Tree health data have not yet been entered or tabulated.

B. El Corte de Madera Open Space Preserve

Tanoak plots were evaluated for disease in October 2011. No symptoms of SOD were seen in either control or treated plots. Tree health data have not yet been entered or tabulated.

Agri-fos bark spray applications

Agri-fos was applied to trees in SFPUC treated plots at Skyline Drive October 31 through Nov 2, and at el Corte de Madera Nov 2 and 3. Mayne Tree applied the Agri-fos at both locations and Ted Swiecki was on hand at the start and end of the applications to provide quality control. Our audit of the SFPUC spray application based on reporting of the material mixed and left over indicated that the applied volume was about 91% of the target amount, which is beyond our typical tolerance of ±5%. In discussing the situation with the crew, the likely problem was that the wand pressure gauge had broken during the application and had not been replaced. That would allow the calibration to slip without being easily noticed. The gauge was replaced prior to the el Corte de Madera application. The audit of this application, based on material mixed and left over, was 95% of the target rate.

A recurring problem with these applications has been the formation of a high amount of unsprayable foam that develops in the spray tank. This problem is most severe in tank sprayers, such as the Mayne Tree sprayer, that use a recirculating action to regulate pressure and agitate the spray solution. The excessive foaming requires periodic draining of the foam, which is essentially unsprayable, thereby increasing the total amount of material and time needed for the application.

Ted Swiecki has contacted Bill Stringfellow (Agri-Fos® technical representative) repeatedly to try to learn if there are any ways to solve this problem. This year Mr. Stringfellow indicated that he had data to support reducing the amount of PentraBark® surfactant used in the application from the label rate of 3.2 fl oz/gal (2.5% v/v) to 1 fl oz/gal (0.79% v/v). This supports our own observations that the 2.5% PentraBark® concentration is too high. Much of the excess surfactant forms a floating, unsprayable emulsion-like layer that separates from the bulk solution. As a result, the concentration of PentraBark® surfactant in the applied solution below the foam layer is really lower than the nominal 2.5% rate.

We used the lower 0.79% PentraBark® rate for the 2011 applications at both tanoak sites and the coast live oak sites discussed below. Even though significant amounts of foam still develop in the tank, the total amount of foam was much reduced. This reduced the amount of down time needed to deal with the foam, and reduced the amount of unsprayable material left over after each application.

2. Management of SOD in canyon live oak and coast live oak using bay removal and Agri-fos treatment

A. Rancho San Antonio Open Space Preserve - coast live oak.

Plot evaluations

We re-evaluated tree health of coast live oaks at on 21 November and 2 December 2011. No new disease was observed at this location. Tree health data have not yet been entered and tabulated. Injected trees were also evaluated for bleeding associated with injection holes. These results are described in the next section.

Agri-Fos applications

Large coast live oaks alongside the Permit Lot at RSA are divided into two Agri-Fos treatment regimes. One set of trees is treated annually with a bark-spray application. The 2001 spray application was completed on 21 November 2011. Ted Swiecki timed the individual spray applications at this site, which were performed by Mayne Tree personnel. The audit of the application indicated that 104% of target amount was used.

The other Agri-Fos treatment used at RSA is stem injection, which is done in alternate years. The last injection treatments were made in January 2011, so re-application is not scheduled until Jan 2013. Our progress report of Jan-May 2011 under Forest Service Agreement No. 10-DG-11052021-214 describes the injections in detail. A brief summary is provided in the following paragraphs.

The initial injections in 2009 were done using the Arborjet injection system. The Arborjet injection system required drilling with a 3/8 inch drill (0.95 cm diameter) through the bark into the wood. Injection was made through a 3/8 inch diameter plastic plug which remained in the wood. None of the injection holes had closed after two years and most injected trees showed bleeding or oozing around some of the old injection holes, and a few had long bark cracks associated with these holes. We felt that the amount of damage associated with the injections was not acceptable for repeated injections over many years, even on a two year re-injection schedule.

We obtained an alternative type of tree injector from ArborSystems (arborsystems.com). for the 2011 injections. This system does not require drilling into the wood. Instead, the injector tips are driven directly into the bark and removed after injection, leaving only a 1-2 mm diameter hole. The injection tips have a number of small holes along the sides which allow for chemical delivery into the inner bark, near the cambium. This system seemed better suited for treating the coast live oaks because it caused smaller wounds and also placed material directly into the inner phloem and cambial region of the lower stem.

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. This is the same rate used with Chemjet injectors by UC Berkeley researchers in Matteo Garbelotto's lab. This required a spacing of 12-14 cm between injection points

Evaluation of the injected trees in November 2011 showed that all of the injected trees were bleeding from the January 2011 injections (Figure 1). Overall, 42% of the injection sites had evidence of bleeding 11 months after the injections.

Given that the injection holes are small, it appears that the bleeding is likely to result from phytotoxicity of the Agri-Fos itself. The ArborSystems injectors are best suited for injecting small amounts of material, since each squeeze of the injector handles delivers about 1 ml of solution. ArborSystems's phosphite formulation, marketed for use with the injector, is the equivalent of non-diluted Agri-fos (45.8% potassium phosphite). For other injector types, this solution is diluted to a 1/3 strength or more dilute solution. We suspected that 45.8% potassium phosphite solution might be too phytotoxic to use without dilution, so we used a 1:1 dilution with water (1/2 strength). Based on our result to date, it seems likely that this concentration is still high enough to cause phytotoxicity. We will continue to monitor the trees to see when the bleeding dissipates, but at this point, the amount of bleeding associated with the injection points appears excessive.

Using a more dilute solution might reduce bleeding. However, we applied an amount that is near the maximum amount possible (6 ml) in each injection site. In some sites, liquid began to leak from the bark around the site. To maintain the same overall phosphite dose per tree, we would need to use more closely spaced injection points with a more dilute phosphite solution. Given that the spacing was already a relatively close 12-14 cm, it is not clear that this would be a viable alternative, especially if bleeding still developed around a significant number of injection holes. At this point, all of the injection options we have tested have had significant shortcomings.

Figure 1. Tree #117 at Rancho San Antonio OSP in November 2011. It shows substantial bleeding from injections made in January 2011 with the ArborSystems injector.

B. Los Trancos Open Space Preserve -canyon live oak.

Plot evaluations

We re-evaluated bay clearances and tree health of canyon live oaks at Los Trancos Open Space Preserve on 31 October, 2 and 17 November, and 9 December 2011. Additional bay removal was performed at Los Trancos during summer of 2011, especially along the Ancient Oaks Trail. As the result of additional bay removal, two study trees were reclassified from controls to the "bay removal only" treatment category. There were no new SOD infections seen at Los Trancos.

Agri-fos applications

Canyon live oaks at Los Trancos were retreated with Agri-Fos on 17 November 2011. Ted Swiecki was present for the entire application. Our audit of the application volume indicated that 104% of the target amount was used, which is within our expected tolerance.

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

San Antonio Open Space Preserve-coast live oak

Area-wide bay removal was used at portions of the Rancho San Antonio Open Space Preserve to protect coast live oaks at risk of developing SOD. Adjacent areas without bay removal are being monitored as controls. Tree health was evaluated on 21 November and 2 December 2011. Data have not yet been entered and tabulated, but we saw no new cases of SOD in the plots.

Monte Bello Open Space Preserve -Shreve oak

Area-wide bay removal has also been used at the Monte Bello Open Space Preserve to protect a unique stand of Shreve oaks. Updated tree health data were collected in July 2011 under MROSD funding (prior to the initiation of this contract). Data for this site have been entered and are graphed below (Figure 2). In the control plots, overall SOD incidence has increased from 16 to 23% of the monitored trees, whereas SOD incidence has remained constant at 14% of the monitored trees in the bay removal plot. In both the control and bay treated plots, one of the late-stage (with beetles and/or *Annulohypoxylon thouarsianum*) SOD-infected trees identified before the study started had died by July 2011. However, new SOD symptoms were only seen in the control plots as of July 2011.

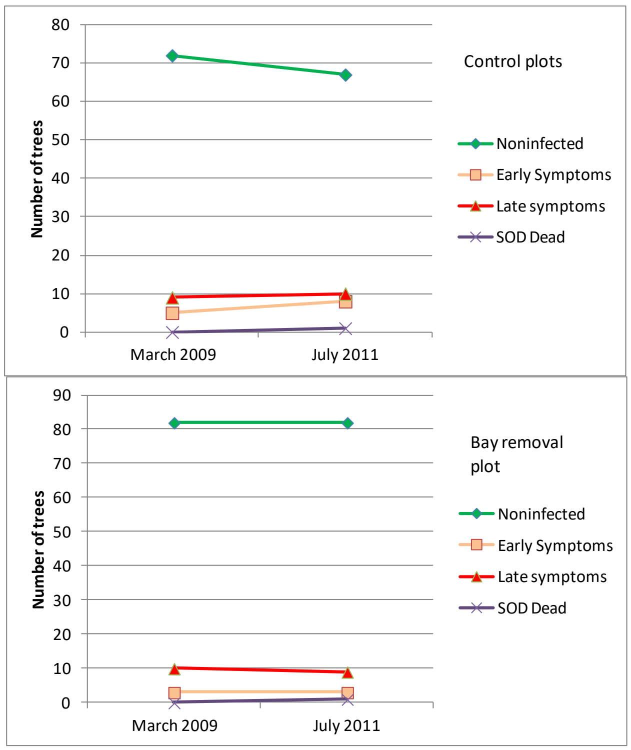

Figure 2. Change in SOD disease status in monitored Shreve oaks within nontreated control plots (top) and an area treated by area-wide bay removal (bottom).

Pulgas Water Temple - coast live oak

The coast live oaks at the Pulgas Water Temple vicinity (SFPUC Peninsula Watershed) were evaluated for disease on 12 August and 2 November 2011. No trees with SOD symptoms were observed among the oaks in the bay removal treatment areas. However, two newly symptomatic trees were observed among the control population. Complete data for this location have not yet been entered and tabulated.

Russian Ridge Open Space Preserve -canyon live oak

At the Russian Ridge Open Space Preserve, bay removal is being used to protect a number of especially large canyon live oaks along a popular trail. Using funding from another source, MROSD staff increased the amount of bay removal in the treatment area in the summer of 2011 at this location. Due to this additional clearing, a few trees previously monitored as controls have been designated as within the bay removal treatment. However, because the additional bay removal did not occur until summer, these trees may still have been subjected to high spore loads in the wet spring of 2011, so they will have to be considered in a separate treatment category.

We remeasured bay-oak clearances and reassessed tree health at Russian Ridge on 9 and 14 December 2011. Data have not yet been entered and tabulated. However, we observed bleeding and ambrosia beetle attack in one trunk of tree RR483, which was in the bay removal area and had a bay-trunk clearance of 5.3 m. Given the advanced symptoms on this trees, it is likely to have been infected for more than a year, i.e., in spring 2010 or earlier. For some of the trees at Russian Ridge, including this specimen, we do not have data on the bay clearance prior to August 2010, so it is not clear whether its bay clearance was previously smaller. The infected portion of the stem is also adjacent to a depression where leaves, including bay leaves, collect (Figure 3, left), which could increase its risk of infection.

Figure 3. Canyon live oaks with new SOD symptoms in 2011 at Russian Ridge OSP. Tree RR483 (left), within a bay removal zone, shows extensive ambrosia beetle boring typical of a late-stage SOD canker. Note that leaves, including bay leaves, collect next to the affected stem. Control area tree RR990 (right) showed minute amounts of recent bleeding (note small dark drips below chipped area). A positive *P. ramorum* isolation was obtained from the chipped portion of the canker.

Within the nontreated area, we also observed a newly symptomatic canyon live oak (tree RR990) with fresh bleeding near the point where bay leaves were in contact with the trunk (Figure 3, right). We were able to obtain a *P. ramorum* culture from this canker, which is one of the few positive isolations obtained from naturally-infected canyon live oaks.

4. Monitor the effectiveness of cut stump herbicide treatments for suppressing bay resprouting in Rancho San Antonio Open Space Preserve and Monte Bello OSP.

In coordination with the tree health assessments noted above, we assessed bay stump resprouting in a sample of bay stumps. Individual stumps are numbered and are relocated by means of distance and azimuth coordinates from tagged oaks. For each stump with live sprouts, we measured the height of the tallest stump sprout and the basal diameter of the largest sprout. With only a few known exceptions, all stumps monitored were treated with glyphosate immediately after cutting.

Glyphosate bay stump treatments at Monte Bello, which occurred originally in winter of 2008-09 were extremely effective in preventing bay sprouting. Most of the treated stumps have died and did not produce shoots. Shoots produced by the few untreated stumps we are monitoring have been browsed and the tallest shoots on these stumps are 23 cm or less in height.

Some mature bays at Monte Bello were net felled but were treated with glyphosate using the hack and squirt methods. Ten of the thirteen bays treated by hack and squirt in winter 2008-09 have died, and an additional tree is mostly dead and has been attacked by ambrosia beetles. However, two bays were missed at that time and were first treated in summer 2009. The hack and squirt treatment had little effect on these trees and they were retreated May 2010. These late-treated bays had still not died by late 2011 and have been treated for a third time by Scott Cotterel (MROSD staff) in Dec 2011.

At Rancho San Antonio, 17 of 67 treated bay stumps (26%) have died. Shoots on remaining stumps have continued to grow, with the tallest heights seen in November 2011, perhaps due to abundant rainfall in spring 2011 (figure 4). Mean height in 2011 (43 cm) is significantly greater than in 2010 (30 cm) and 2009 (22 cm) (Tukey HSD alpha=0.05). Nearly all bay sprouts have been browsed, but the tallest shoots are typically in areas where browsing is reduced (e.g., due to slope or interfering vegetation). As shown in figure 4, shoot heights are highly variable between stumps.

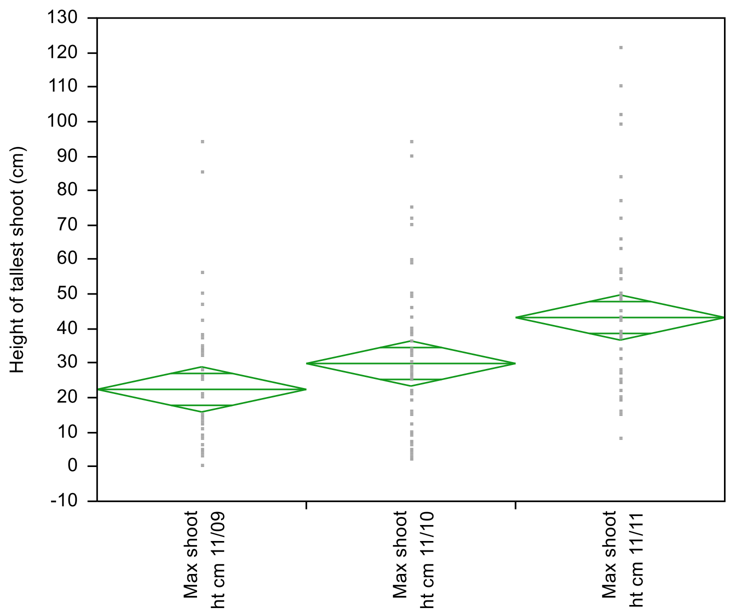

Figure 4. Heights of shoots from glyphosate-treated bay stumps at the Rancho San Antonio permit lot area. Each dot represents tallest sprout on a stump. The center line of each diamond represents the mean height of tallest shoot; the vertical extent of each diamond represents the 95% confidence interval based on a pooled variance for all segments.

5. Re-evaluate long term monitoring plots in Marin, Napa, and Sonoma counties

Plots were evaluated between 26 August and 19 September 2011. Disease status data has been entered and tabulated (figure 5). Other collected data (e.g., failures) have not yet been entered. Overall, the rainy spring weather of 2010 and 2011 has not yet resulted in a net increase in the number of coast live oaks with SOD symptoms. This suggests that new infections in 2010 were minimal and/or new infections from 2010 were associated with a relatively long latent period (>1.5 years), possibly due to low inoculum levels. Given that the latent period for SOD symptom development is usually at least one year in oaks, we did not expect to see symptoms associated with spring 2011 infections in September 2011.

For tanoak, the situation was somewhat different. The percentage of tanoaks with SOD symptoms started to increase in 2010 after leveling out between 2007 and 2009. Hence, it appears that there was a rather short lag between favorable disease conditions and the development of SOD symptoms in these tanoaks. However, the residual tanoaks in these plots are mostly relatively small diameter trees. This may account for the fact that the trees seem to show a relatively short latent period.

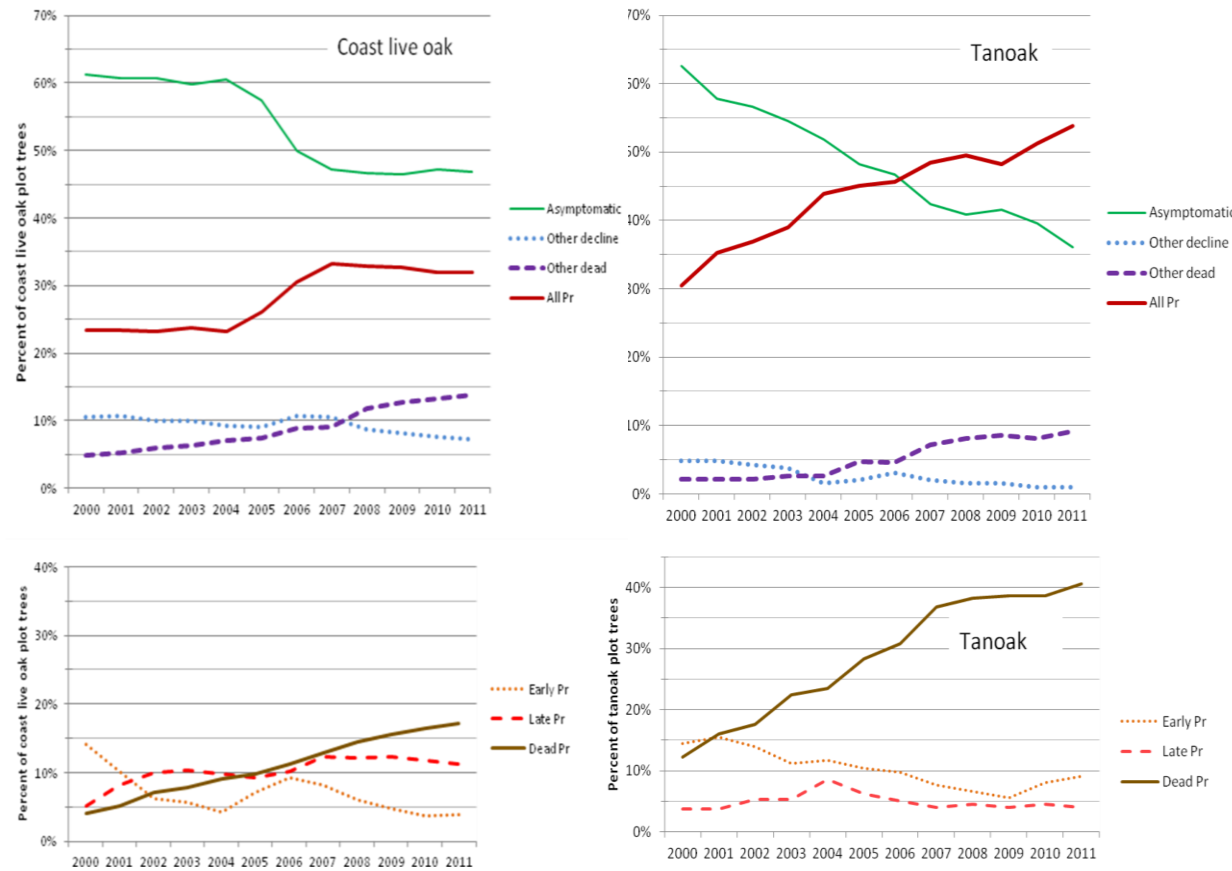

Figure 5. Disease progress in long term monitoring plots in Marin Napa and Sonoma county evaluated in September of each year.

6. Canyon live oak – improving SOD diagnosis - Skyline Ridge Open Space Reserve, Rattlesnake Point

We evaluated inoculated trees on 6 July 2011, about 12 months after inoculation, and on 9 December 2011, about 18 months after inoculation. The inoculation was performed prior to the start of this contract in July 2010.

Methods. Our progress reports produced under our previous contract described the procedures we used to inoculate and reisolate *P. ramorum* from canyon live oak in the field at Rattlesnake Point. We inoculated nine canyon live oaks in each of the two areas at this location. The lower plot area is a closed canopy stand dominated by relatively tall canyon live oaks with small, often thinning crowns, some of which are partially overtopped. This plot area has some intermixed tanoak and a few bay. Some of the bays have been removed since the inoculations were done. The upper plot area was a restoration planting, which also has a closed canopy, but trees are much shorter with wider crowns. Most canopies are relatively dense, and the lowermost branches are mostly dead or dying due to shading out. The planted stand includes canyon live oak, shreve oak, and a few tanoaks. The seed source(s) are unknown, but are probably from the Peninsula.

We also inoculated two Shreve oaks at the upper restoration site to act as positive controls. Each tree was inoculated with two different local *P. ramorum* isolates and a control (sterile agar only) inoculation. The three inoculation points were spread out as far as possible around the circumference of the trees, which averaged about 25 cm DBH.

Symptoms. We found that relatively few *P. ramorum* bole cankers on inoculated canyon live oaks developed visible bleeding (Table 2). Overall, some bleeding was associated with 9 of 36 (25%) individual inoculation sites through Dec 2011, but recent bleeding was never seen at more than four inoculation sites (11%) at any given observation point. Most trees with bleeding symptoms showed miniscule amounts of bleeding that could only be seen on close inspection. Only one tree (168, upper plot), which developed a massive canker by November 2010, had enough bleeding to be noticeable at any distance. In trees that showed bleeding, the period of active bleeding was fairly short. Only two trees (168, 169) have shown active bleeding in successive observations (Table 2). Both the amount of bleeding and the period of active bleeding were much less than is typical in SOD-infected coast live oaks.

These results are consistent with our field observations showing that evidence of bleeding is very uncommon in naturally infected trees. Few SOD cankers on canyon live oak develop bleeding. Among those that do, the amount is very small and bleeding occurs for only short periods. As a result, evidence of past bleeding may wash off over time leaving no clear trace even among trees that did bleed. As a consequence, SOD is very difficult to find and confirm at early stages in naturally-infected canyon live oaks. Nonetheless, as discussed above, we have been able to detect and confirm some early-stage SOD cankers in naturally-infected canyon live oaks.

Table 2 . Evidence of recent bleeding beyond the inoculation plug for canyon live oak and Shreve oak trees at inoculation points through 12/9/11. Note that the specific trees showing bleeding vary over time.

		Recent bleeding present at date assessed						
Species	**Totals**	**11/1/10**	**12/10/10**	**2/4/11**	**4/6/11**	**4/30/11**	**7/6/11**	**12/9/11**
Canyon live oak								
Trees	18	3	3	0	3	3	1	2
(tag #)		(159,165,168)	(162,168,171)		(168,169,172)	(159,169,172)	(168)	(161,162)
Inoculation points	36	4	4	0	3	3	1	2
Shreve oak								
Trees	2/	0/	1/	0/	2/	2/	2/	1/
Inoculation points	4	0	1	0	4	4	4	1

One of the four inoculation points on the two Shreve oaks showed bleeding by 12/10/10. Multiple bleeding spots were associated with all four Shreve inoculations by April 2011. By December 2011 active bleeding was evident on only one of the Shreve oaks. Although the bleeding on the inoculated Shreve oaks was also limited, it was much more extensive than seen on the canyon live oaks.

Through the end of December 2011, the canopies of all trees were green and showed no significant thinning or dieback. Tree 168 in the upper plot, which has a massive canker, had slightly yellow leaves in the canopy of the inoculated stem. One canyon live oak in the upper plot (#171) was heavily infested with ambrosia beetles in a broad area around both *P. ramorum* inoculation points in July 2011. By December, newly emerging fruiting bodies of *Annulohypoxylon (Hypoxylon) thouarsianum* were visible on this tree. In addition, one Shreve oak (#173) showed evidence of ambrosia beetle boring where the canker had been previously excised.

A common symptom which has developed on the trees is bark cracking near the inoculation point, as shown below (Figure 6). This reaction is presumed to be a resistant reaction, indicating that the tree has been able to stop progression of the fungus in the cambium and develop healthy callus tissue under the bark. Such resistant reactions, where they occur, should help infected trees to recover. Therefore removing bay from around infected canyon live oaks, such as those at Los Trancos, should help recovery by reducing the number of new infections which might develop. Multiple infections over time would challenge the ability of the tree to mount resistant reactions.

Figure 6. Bark cracking around inoculation point (dark circle - about 9mm in diameter) in tree 166. In the field callus is visible under the cracked bark on both sides of the plug. This callus symptoms has been observed on canyon live oak growing under bay heavily infested with *Phytophthora ramorum* (right photo Russian Ridge), and we believe these callus ridges are a resistant reaction to infection.