Sudden Oak Death Management and Monitoring in the Bay Area
Forest Service Agreement No. 10-DG-11052021-214

Progress report June-Dec 2010

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Objectives for the project are listed below. This contract continues management and monitoring projects that began in 2008 under contract 08-DG-11052021-144. 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 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	Monitor the effectiveness of hack and squirt herbicide treatments for killing large bay in bay removal disease suppression projects at Monte Bello OSP
6	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.

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Overview

Major project activities during the period occurred for objectives 1, 2,3,4, and 6:

Annual Agri-fos spray applications were made at the following locations in November 2010:

-tanoak in plots at MROSD El Corte de Madera Open Space Preserve and SFPUC lands in the Peninsula Watershed near Crystal Spring Reservoir (Skyline Drive)

- canyon live oaks at MROSD Los Trancos Open Space Preserve

- coast live oaks at MROSD Rancho San Antonio Open Space Preserve

We re-evaluated disease status of monitored trees at the following locations:

-tanoak in Agri-fos treated and control plots at MROSD El Corte de Madera Open Space Preserve and SFPUC lands in the Peninsula Watershed near Crystal Spring Reservoir (Skyline Drive),

-canyon live oaks treated with bay removal and Agri-fos and control trees at MROSD Los Trancos Open Space Preserve,

-coast live oaks treated with bay removal and Agri-fos and control trees at MROSD Rancho San Antonio Open Space Preserve.

-area wide bay removal and control plots coast live oaks at MROSD Rancho San Antonio Open Space Preserve.

We recorded data on bay sprout regrowth at MROSD Rancho San Antonio Open Space Preserve.

We established plots to monitor the effectiveness of bay removal in preventing SOD on canyon live oaks at MROSD Russian Ridge Open Space Preserve. We obtained the first positive isolation of *P. ramorum* from a canyon live oak bole canker during this activity.

We collected data on long-term SOD monitoring plots established in 2000 (Marin, Sonoma, Napa Co.).

Koch's Postulates were completed for *P. ramorum* on canyon live oak and Shreve oak.

The five projects set up on MROSD lands are summarized in Table 1. Because all of the sites are in the vicinity of the SOD management studies that we are conducting on the SFPUC watershed, it has been possible to coordinate some of the plot work at SFPUC sites with work at MROSD sites, reducing total travel-related costs. Details of progress over the reporting period are discussed below.

Table 1. SOD management studies initiated on MROSD lands in 2008/2009/2010.

Open Space Preserve	**Locality**	**Host species present** (bold= primary species)	**Treatment(s)**	**Treated area sample size**	**Untreated area 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	158 stems	164 stems
Monte Bello	Skid Road trail gate (MB06)	**shreve oak**, canyon live oak	areawide bay removal: Dec 2008, Mar 2009	97 stems	86 stems
Rancho San Antonio (RSA)	permit lot area	**coast live oak**	Localized bay removal and Agri-Fos injection: Nov 2008, planned for Jan 2011. Localized bay removal (Nov 2008) and Agri-Fos stem spray application: Jan 2009, May 2009, Nov 2009, Nov 2010 Areawide bay removal only: Nov 2008	9 stems* 14 stems* 42 stems	61 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 Localized bay removal only: Dec 2009, April 2010	16 stems 20 stems	31 stems
Russian Ridge	Near Ancient Oaks Trail	**canyon live oak**	Localized bay removal only: Dec 2009, Sep 2010		

* 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.

El Corte de Madera Open Space Preserve: tanoak - Agri-fos stem application

The Agri-fos treated area at the El Corte de Madera OSP is about 0.36 ha (0.9 acre) (*figure 1*). The 4 matched control plots on the periphery of the treated plot are similar in species composition and stem size distribution to the treated plot. The total area (0.36 ha) and stem density within the control plots (*table 1*) are virtually identical to that of the treated plot. All tanoak stems and a few Shreve oaks and canyon live oaks that are within the plots were re-evaluated for disease presence in July 2010. No disease was seen in the treated plots. Although there were bleeding cankers on a few of the trees in the control plots, isolations for *P. ramorum* from all symptomatic trees were negative.

The treated plot is on a fall application schedule. Agri-fos was reapplied in Nov 2010. As in other related studies, the application was made by banding the material high on the bole (to a height of about 5 m) using a high reach spray wand. About 144 L (38 gal) of spray solution is required to treat the plot.

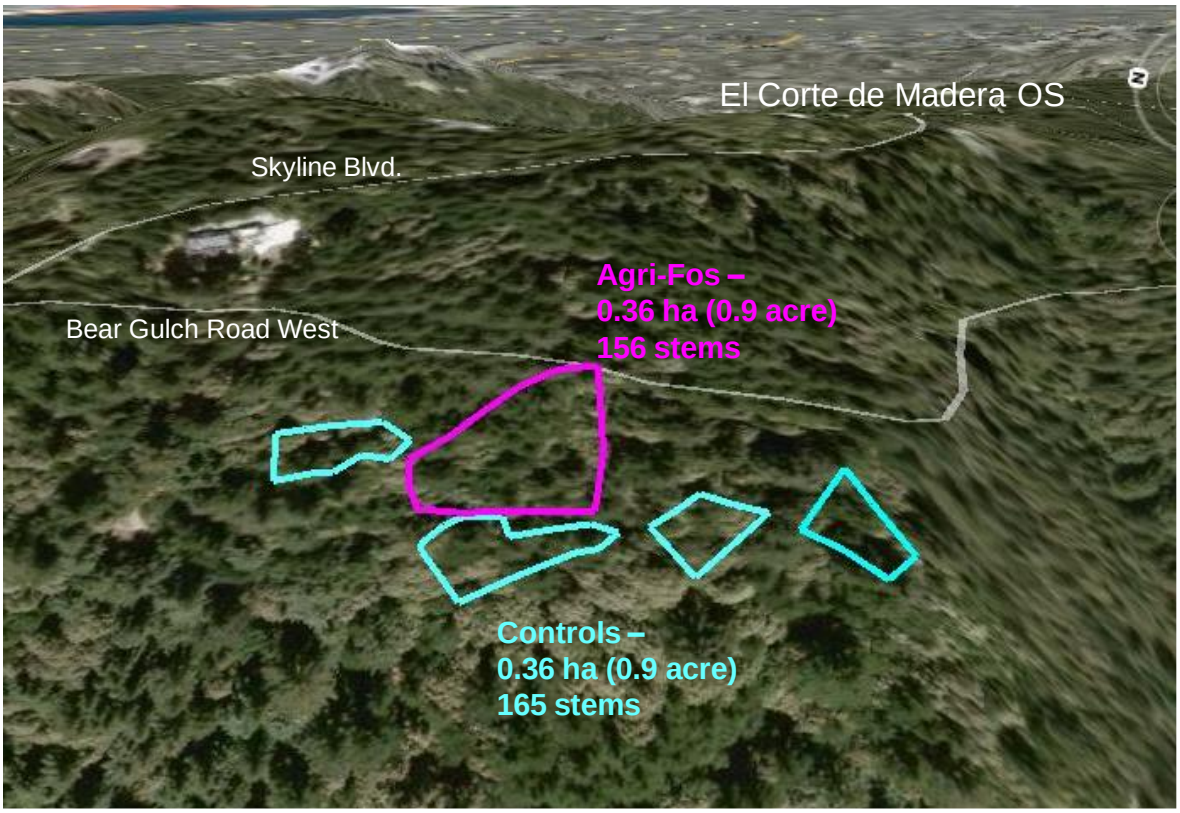

Figure 1. Aerial image of Agri-Fos treated area (magenta outline) at the El Corte de Madera OSP. Cyan polygons are areas where control trees are located.

Rancho San Antonio Open Space Preserve

Due to the particular constraints and opportunities at this heavily used open space, we are testing multiple SOD management techniques at this location (Table 1). Locations of treated and untreated control plots are shown in Figure 2.

Coast live oak - Agri-fos treatment and localized bay removal

Large high value oaks near a trail and creek were treated by conducting localized bay removal to the degree possible and treating the trees with Agri-fos either by high bole spray application (11 stems initially) or injection (13 stems initially) using the Arborjet "Tree I.V." injection system (under our previous contract 08-DG-11052021-144). Bay removal was limited in this area because it was impractical and undesirable to remove many of the large bays along the creek. Bay removal in the area was primarily limited to the removal of a dense understory of small bays, removal of a few larger bays, and pruning off some stems and branches of bays that were left in place.

Agri-fos was reapplied in November 2010. The Agri-fos treatment schedule has been coordinated with the schedule at El Corte de Madera and Los Trancos to minimize costs and improve efficiency. Applications made to date are summarized in Table 1.

Among this set of coast live oaks, one of the sprayed trees was removed by MROSD as a potentially hazardous tree in 11/09. One injected stem of a multistemmed tree failed in 2009. This changed the geometry of this large oak relative to the creek to make spray application feasible. Starting with the fall 2010 application, we switched the three remaining stems of this tree to spray application. As a result, the number of injected stems changed from 13 to 9 and sprayed stems from 11 to 14.

Coast live oak - area-wide bay removal

In other portions of the RSA site where it was feasible, we are testing area-wide removal of bay. Trees in these bay removal areas (Figure 2) are not treated with Agri-fos. In the area closest to the trees treated with Agri-fos, equipment access was limited due to the creek and hill slope, so cut material was left in place (lop and scatter). The other bay removal area was easier to access, so cut bays were removed and chipped. Bay removal treatments were mostly completed in late 2008, but some follow-up work was done in January 2009 to expand the treated area.

Monitoring results

In November 2010, we remeasured oak trunk-bay foliage clearances around all monitored oaks and re-evaluated trees for SOD symptoms. No new SOD symptoms were identified among the trees. Analysis of oak-bay clearance data will be reported in the next progress report. One injected tree has developed thinning and top dieback that we had attributed to decay. In fall 2010, we observed an *Inonotus dryophilus* conk near the base of the scaffold showing the most severe dieback.

Among the injected trees, none of the injection holes had closed over after two years. Most injected trees showed some recent bleeding or oozing around some of the old injection holes, and a few had long bark cracks associated with these holes.

Given that the injected trees were due to be injected again, we felt that the amount of damage associated with the previous injection was not acceptable. After making some inquiries, we obtained an alternative type of tree injector for testing from ArborSystems. They provided a Direct-Inject QC injection system with Portle injection tips for testing. The injector tips are driven directly into the bark and leave a much smaller wound than

the Tree IV system used previously. The use of the ArborSystems Direct-Inject equipment to retreat injected trees will be described in the next progress report.

We remeasured the maximum heights and diameters of bay resprouts from a previously monitored set of glyphosate-treated stumps in the treated area. Although the data have not yet been analyzed, sprout suppression has generally been good to date.

Figure 2. Aerial image of treated and control areas at Rancho San Antonio OSP. Magenta polygons outline areas where both bay removal and phosphite application have been used. Orange polygons are areas where bays have been removed but phosphite has not been applied. Cyan polygons are areas where control trees are located. The Foothills Ranger Station is visible at the upper left corner or the image.

Los Trancos Open Space Preserve

Canyon live oak - Agri-fos treatment and localized bay removal

We initiated management studies to determine whether techniques used to protect coast live oaks from SOD would be effective on canyon live oak at the Los Trancos preserve in 2009 under our previous contract. Only localized bay clearing was feasible in the project area due to the presence of many large bays. Localized bay removal only is being used around 9 tagged canyon live oak stems. An additional 16 stems, a number of which are very large trees, are also being treated with Agri-fos using the high bole bark spray application. Several of these stems currently have old cankers, but are being treated because the trees have been identified as having high aesthetic, historic, and/or cultural value.

Agri-fos was reapplied to treated trees in November 2010. The Agri-fos treatment schedule for these trees is also coordinated with applications at RSA and El Corte de Madera for greater efficiency

In November 2010, we remeasured oak trunk-bay foliage clearances around all monitored oaks and re-evaluated treated and control trees for SOD symptoms. No newly-symptomatic trees were observed at that time.

Russian Ridge Open Space Reserve

Canyon live oak – localized California bay removal

The Trail of the Ancient Oaks is a highly-visited trail in the popular Russian Ridge Open Space Reserve that winds past numerous giant canyon live oaks. Many of these trees are surrounded by California bay. Due to more difficult access at this site and the sheer size and number of trees present, Agri-Fos application was not a cost-effective option for this area. However, localized bay removal near trees closest to the trail was a viable SOD management option (Figure 3).

Initial bay removal was conducted in December 2009. Small bays were cut by CCC crews and an arborist contractor (Mayne Tree) was used to cut down some larger trees that were too difficult for the CCC crews to handle. Stan Hooper from MROSD marked additional bays for removal in August. In our site assessments in August 2010, we marked additional bays for removal to improve clearances. Additional bay removal was conducted in September 2010.

We collected baseline data on disease status of trees in the bay removal area and established controls beyond the bay removal areas in August 2010. We also assessed oak-bay clearances of all treated and control trees at that time. Clearance data will be updated for trees around which additional bays were removed at our next assessment.

Canyon live oak – improving SOD diagnosis

While we were evaluating trees for use as untreated controls at Russian Ridge in August 2010, we were also actively looking for early SOD symptoms on canyon live oak. Conditions had been favorable for *P. ramorum* sporulation in spring 2010, so the odds of finding an early stage infection were better at that time than in our previous sampling. We also had a better search image for internal canker symptoms based on the detached log study (see following section).

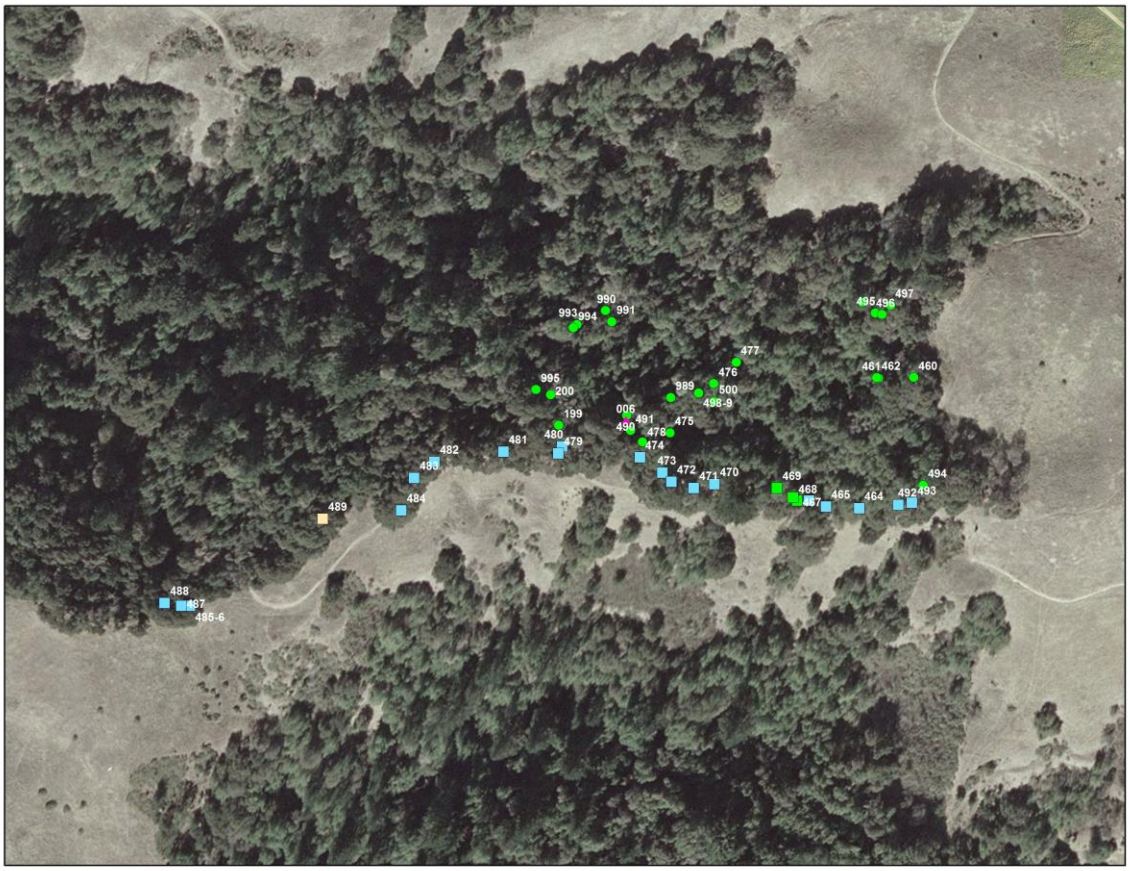

Figure 3. Canyon live oak treated using localized bay removal (squares) and nontreated controls (circles) in the Russian Ridge OSP. Tree 475 near center of image is the location of the canyon live oak with the *P. ramorum*-positive canker noted below.

We isolated from a number of promising cankers and finally recovered *P. ramorum* from a relatively small canker on a canyon live oak near symptomatic bays (figure 4). This particular canker showed very small amounts of bleeding. Our isolation efficiency from this canker was rather low: only two positive chips were obtained on two plates of samples. This was the first (and to date, the only) isolation of *P. ramorum* from a bole canker on a naturally-infected canyon live oak tree.

Skyline Ridge Open Space Reserve

Canyon live oak – improving SOD diagnosis

Our previous work for MROSD and under FS-FHP contract 08-DG-11052021-144 had indicated that *P. ramorum* caused bole cankers on mature canyon live oak trees. This conclusion was based on epidemiological evidence and a positive PCR result from a sampled canker. However, all field isolations from canyon live oak bole cankers conducted through 2009 had been negative for *P. ramorum.* However, all canyon live oak SOD cankers that we had observed through that point were at the late stage, i.e., with beetle boring and *Annulohypoxylon thouarsianum*. Given that the last likely infection period had been in spring 2006, it is not surprising that infections had advanced to the late stage by 2008 and 2009. This clearly had adverse effects on isolation efficiency, and

also left a critical gap in our knowledge of symptomatology in this species. A major question was whether bleeding cankers actually develop on canyon live oak at the early infection stage.

Figure 4. Canyon live oak with *P. ramorum*-positive canker. Upper right, external symptoms of canker, lower right, canker appearance after outer bark was cut away.

To improve our ability to diagnose SOD, and to provide more definitive confirmation that canyon live oak trees are SOD canker hosts, we have undertaken two studies in cooperation with Kamyar Aram of the Rizzo lab. The first study, reported previously, was conducted in November 2009. Detached canyon live oak logs were inoculated with *P. ramorum* under lab conditions, following RAPRA procedures. Cankers developed on inoculated logs and we recovered *P. ramorum* from the cankers. This test provided further evidence of pathogenicity, as well as the first look at the internal appearance of known cankers on this species. However, the detached log assay does not tell us what types of external symptoms develop on intact trees in the field.

To address this and other questions, we collaborated with the Rizzo lab to inoculate canyon live oaks with *P. ramorum* in the field. Kamyar Aram applied for and received a permit from CDFA for field inoculations. This study had several goals. First, it would allow us to complete Koch's postulates for this species. Second, it would allow us to address a number of data gaps related to the diagnosis of SOD in this species, including early symptom development, relative susceptibility, the rate of symptom development, and changes in isolation efficiency from cankers over time. This information is needed to allow us to assess the effectiveness of SOD treatments.

In mid-July 2010, we and Kamyar looked at several potential sites for this study with Cindy Roessler of MROSD. We identified suitable trees for inoculation at the Skyline Ridge OSP. The area, referred to as Rattlesnake Point, is close to the Skyline Ranger station and has easy access. Part of the site is a natural forest, the other part is an old restoration planting at the site of a former Christmas tree farm. The seed source for restoration planting (now overly dense and in need of thinning) is of uncertain provenance and possibly not closely related to the adjacent natural stand.

In late July 2010, we inoculated nine canyon live oaks in each of the two portions of the site. We also inoculated two Shreve oaks at the 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 were evaluated periodically over the next few months. Neither of the Shreve oaks and almost none of the canyon live oaks showed any bleeding associated with the inoculations. Only a few canyon live oaks showed a tiny amount of bleeding. These results suggest that early symptoms of infection on canyon live oak may be obscure at best and may be entirely cryptic in many cases.

In December 2010, we conducted our first sampling of inoculated trees. We shaved the bark away to expose cankers on one Shreve oak and six of the canyon live oaks, three from each site. The Shreve oak had been entirely girdled by the inoculations. All canyon live oaks had large cankers at inoculation points. The one canyon live oak that had shown the most bleeding was mostly girdled. However, even the amount of bleeding on that tree was miniscule compared to what is typically see in affected coast live and California black oak.

We successfully reisolated *P. ramorum* from all sampled cankers on both species. We took detailed data and photos during the sampling, which will provide data on which sample locations were most successful. In this first round of sampling, recovery was very poor from the deepest tissue samples collected near the cambium in the outer discolored sapwood.

The successful reisolations effectively complete Koch's postulates for SOD on canyon live oak. In addition, the reisolation of *P. ramorum* from the inoculated Shreve oak is the first formal completion of Koch's postulates for this species.

Collect data in long-term SOD monitoring plots

We collected data in our 150 long-term SOD monitoring plots in Marin, Napa, and Sonoma counties in September and October 2010. We collected data at the 12 locations over six days. Data evaluated included current *Phytophthora ramorum* disease status; the amount of the stem girdling by *P. ramorum,* beetles, and *Annulohypoxylon thouarsianum*; canopy dieback; and the occurrence and characteristics of new failures. We entered and tabulated the data. As shown in figure 5 below, the number of infected tanoaks increased slightly in 2010, but there was no net change in disease incidence in

coast live oak. Monitoring data from 2011 will be needed to see if SOD levels in susceptible oaks have increased in connection with the 2010 (and 2011) rainy seasons.

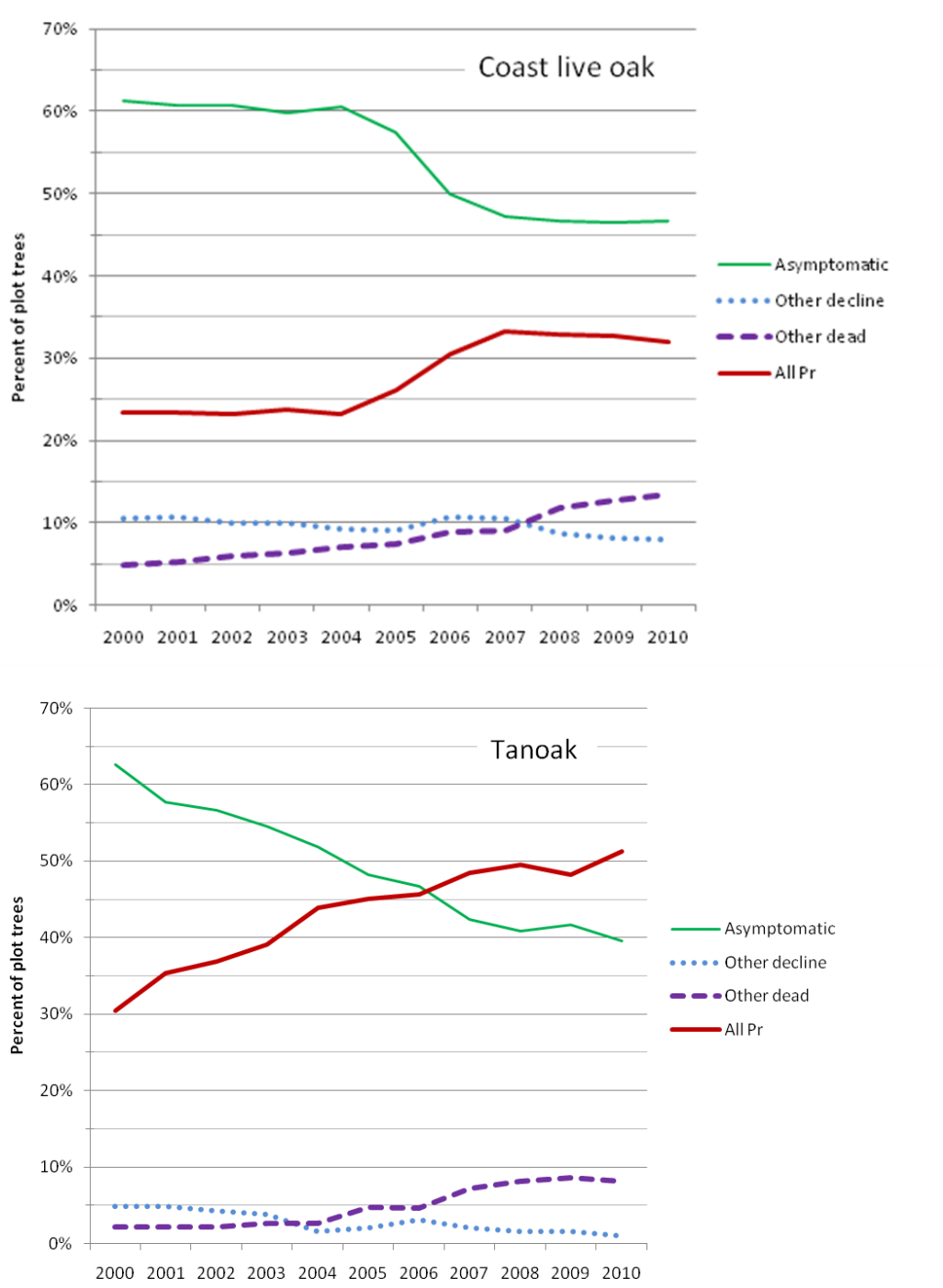

Figure 5. Overall disease level changes 2000-2010 for coast live oak and tanoak in plots located in Marin, Napa, and Sonoma counties.