Phytophthora ramorum Canker (Sudden Oak Death) Disease Risk and Progress in Coast Live Oak, 2000-2012[1]

Tedmund J. Swiecki[2] and Elizabeth Bernhardt[2]

Abstract

From 2000 through 2012, we collected annual observations on disease symptoms and stand conditions in 128 coast live oak plots in forests affected by sudden oak death (SOD), caused by the introduced pathogen *Phytophthora ramorum*. Elevated rainfall in one or both of the previous wet seasons was associated with pulses of new infections. However, persistent differences in infection rates between nearby locations and among plots within locations show that tree and site specific factors influence disease risk on the local scale. Because California bay is the primary source of *P. ramorum* spores in these affected forests, variables describing the proximity and density of California bay in the local oak neighborhood are the strongest predictors of disease risk. Tree growth rate and bark characteristics are also predictors of disease risk. Faster-growing, more dominant trees had elevated SOD risk whereas trees declining from other diseases had reduced SOD risk. Coast live oaks with SOD followed one of several disease progress trajectories, ranging from rapid decline to disease remission. Extensive initial trunk girdling by cankers was associated with rapid decline. More than half of the trees that developed symptoms between 2001 and 2010 had inactive or undetectable cankers by 2012.

Key words: California bay, disease remission, *Quercus agrifolia*, resistance, risk model, susceptibility, symptoms, *Umbellularia californica*

Introduction

In 2000, the exotic plant pathogen *Phytophthora ramorum* was identified as the cause of sudden oak death (SOD), a lethal trunk canker disease. In California, coast live oak (*Quercus agrifolia*), California black oak (*Q. kelloggii*), canyon live oak (*Q. chrysolepis*) and tanoak (*Notholithocarpus densiflorus*) are among the species that have been most severely affected to date (Garbelotto and others 2001, Rizzo and others 2002, Swiecki and others 2013). Mycelium of *P. ramorum* colonizes and kills phloem tissues in the bark and can spread to the cambium and outer xylem in these susceptible species. Infections cause bleeding bark cankers that can expand over time and eventually girdle susceptible trees. The sapwood-decaying fungus *Annulohypoxylon thouarsianum*, ambrosia beetles (*Monarthrum* spp.), and oak bark beetles (*Pseudopityophthorus* spp.) commonly attack *P. ramorum*-infected trees and contribute to tree decline (Garbelotto and others 2001).

We initiated a long-term, permanent-plot study in September 2000 in areas where SOD had already become established to evaluate disease risk factors and monitor disease outcomes over time. Original plot setup and all plot data collection between 2000 and 2012 were performed by the authors. This has allowed for a high degree of uniformity in assessment methodology and has increased our ability to detect and track trends in disease progress. Based on our field observations, we initiated data

[1] An abbreviated version of this paper was presented at the Seventh California Oak Symposium: Managing Oak Woodlands in a Dynamic World, November 3-6, 2014, Visalia, California.

[2] Phytosphere Research, 1027 Davis Street, Vacaville CA 95687. (Phytosphere@phytosphere.com).

collection on additional variables not considered in the original study design. These variables have been used to improve risk models to predict disease development. In this paper we discuss risk models and disease outcomes in coast live oaks.

Methods

Plot selection

During September 2000, we established circular 8 m radius (0.02 ha) plots at nine coast live oak study locations in Marin County and one in Napa County. Plots were established in areas where *P. ramorum* was prevalent. Each plot was centered around a case or control tree. Case trees had only early symptoms of *P. ramorum* canker (bleeding cankers), with the exception of nine cases that had minor amounts of beetle boring or a few small *Annulohypoxylon thouarsianum* stromata, typically on a single scaffold or on a localized portion of the bole. Control trees were lacking any *P. ramorum* canker symptoms. We established 128 coast live oak plots (53 cases, 75 controls). The locations of study sites and methods used to select plots have been previously described in detail (Swiecki and Bernhardt 2002).

Disease ratings

The authors evaluated trees in plots each year from 2000 through 2012 in September or October. Trees that had at least one stem 3 cm in diameter at 1.37 cm height (DBH) were included in the tree size class.

For coast live oak trees in each plot, we noted whether *P. ramorum* canker symptoms were present on the main stems and the stage of disease development. Trees were classified as having early symptoms if only bleeding cankers were present. Late disease symptoms were defined as including cankers and *A. thouarsianum* stromata and/or beetle boring. Trees killed as a result of extensive *P. ramorum* cankers were classified in the dead *P. ramorum* symptom class. Symptomatic trees that later stopped bleeding and showed no other evidence of disease progress for at least 2 consecutive years were classified as inactive infections (early or late depending on other symptoms). Trees in which inactive cankers became undetectable were rated as asymptomatic. Severe decline (tree death likely within 10 years) and death due to agents other than *P. ramorum* (primarily canker rot fungi) were also evaluated for each oak tree.

Disease ratings were based primarily on visual assessments. When necessary, we chipped away outer bark to expose and sample cankers, but this was minimized to avoid possible effects on future observations. To culture the pathogen from sampled cankers, we placed bark tissue pieces from canker margins into PARP semi-selective media (Erwin and Ribeiro 1996) to isolate the pathogen. In a few instances, we resampled cankers that had yielded negative results in previous years.

Trunk girdling by *P. ramorum* cankers, *A. thouarsianum*, and beetles were scored by estimating the percent bole circumference girdled as if all affected areas were projected onto the same cross section. A pretransformed 0 to 6 scale was used for these and most other visual estimates of percentages: 0 = not observed, 1 = trace to 2.5 percent, 2 = 2.5 to 19 percent, 3 = 20 to 49 percent, 4 = 50 to 79 percent, 5 = 80 to 97.4 percent, 6 = >97.5 percent. We noted whether cankers had oozed during the previous year. Canopy dieback and the abundance of unweathered bark fissures in the lower bole were also rated using 0 to 6 scale.

The influence of California bay (*Umbellularia californica*) on disease risk was assessed by recording the number of bay stems (3 cm DBH or greater) in each plot and bay canopy cover in the plot (0-6 scale). For each oak in the plot, we also measured the minimum bay foliage-oak trunk clearance, and bay canopy cover within 2.5 m and 5 m of the oak trunk using a 0-4 scale (0 = no bay canopy, 1 = 1 to 25 percent cover, 2 = 26 to 50 percent cover, 3 = 51 to 75 percent cover, 4 = more than 75 percent cover). Presence of bay within 5 to 10 m and 10 to 20 m from each oak trunk was also recorded.

Results

Figure 1 shows the overall disease status of study trees from 2000 through 2012. Slight declines in SOD incidence after 2003 and 2007 are due to complete symptom remission in some trees. In periods when disease levels were static, the percentage of trees with early symptoms declined as trees advanced to late or dead stages (fig. 1 bottom).

Overall mortality from SOD increased in a linear fashion between 2000 and 2012, as has mortality due to other factors. Total SOD mortality was slightly below mortality due to other factors in 2000, but has exceeded non-SOD background mortality from 2002 onward. Live trees with SOD symptoms were at least two to three times more numerous than trees declining due to other factors over the entire study period.

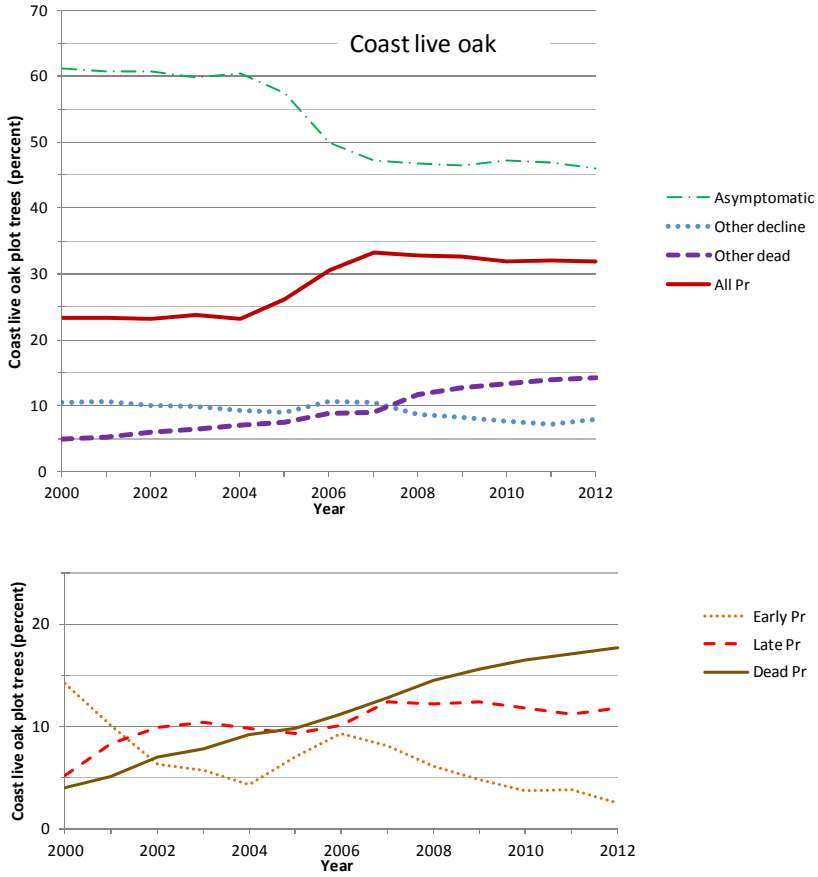

Figure 1—Disease status of 655 coast live oak trees from 2000 through 2012. Top graph shows percentages of trees with SOD symptoms (all Pr) as well as asymptomatic trees and trees declining or dead due to causes other than SOD. Bottom graph shows percentages of trees in various *P. ramorum* canker stages (early, late, or dead).

Between 2001 and 2012, 107 trees developed initial SOD symptoms. This represents 21 percent of the noninfected trees present in 2001. The number of newly symptomatic trees varied widely from year to year, with a large pulse of new infections starting in 2005 and smaller pulses in 2003 and 2012 (fig. 2). No newly diseased trees were observed in 2009 and only one was seen in 2008.

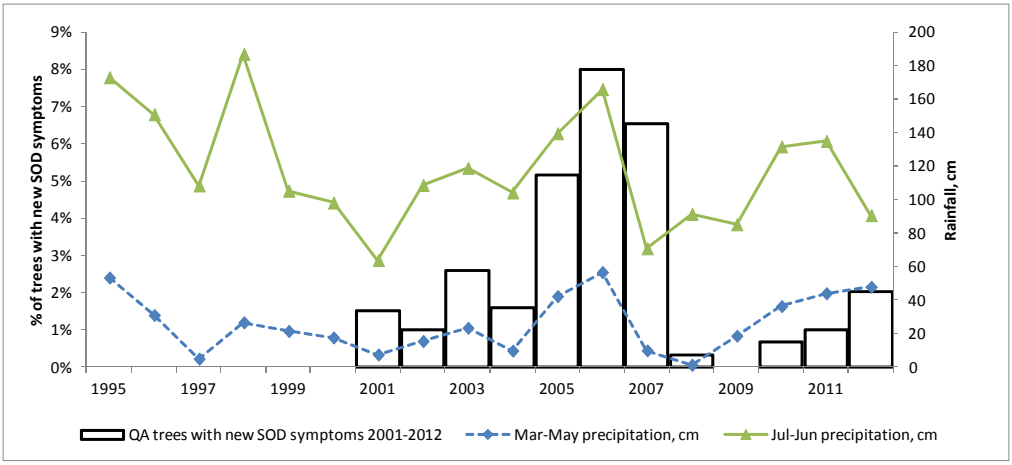

Figure 2—Percent of new SOD infections observed each year (2001-2012) and precipitation (1995-2012) for Kentfield (Marin County). Annual rainfall (dashed) includes period from July of the previous year to June of the year shown.

The number of newly symptomatic (fig. 2) trees seen in a given year was correlated with weather conditions favorable for disease cycling and infection. Both current year and previous year rainfall totals were significant predictors of the number of coast live oaks with new SOD symptoms in Poisson models. The best model overall for predicting new infections over the observation period included both previous and current year seasonal rainfall (model P <0.0001).

Disease trajectories

Through repeated observations of trees with *P. ramorum* canker symptoms, we found that most symptomatic trees followed one of the following disease progress patterns.

Rapid decline

Trees developed extensive P. *ramorum* bole cankers that were heavily colonized by secondary organisms (beetles and *A. thouarsianum*) usually within a year of canker development. Trees died within 2 years of initial symptoms without significant canopy thinning, typically with rapid drying and browning of the entire canopy.

Rapid decline developed most commonly in trees that had extensive cankers at initial symptom onset. For trees that developed initial symptoms between 2000 and 2010, the average initial *P. ramorum* canker girdling rating was significantly higher (Tukey-Kramer HSD $p < 0.05$) in trees that died within two years (mean 5.1, n=15) than for those that died 3 to 11 years after initial symptom development (mean 2.3, n = 10) or were still alive in 2012 (mean 1.9, n = 43).

Slow decline

Canker development was moderate to extensive, sometimes increasing gradually over several years. Invasion by secondary organisms often occurred within 2 years of canker appearance but was sometimes delayed. After a few years, cankers commonly developed callused margins. The canopy showed slow progressive thinning and decline as bole girdling approached or exceeded 50 percent. Most oaks with this

trajectory survived for 4 to 6 years but many survived for 10 years or more. Tree with extensive cankers were often killed by bole failure before canopy death occurred.

Partial to complete symptom remission

Relatively small cankers ceased bleeding or expanding after 1 to 2 years. Cankers dried out and became inactive. Larger cankers developed callus around the margins that eventually closed the cankered area. Small cankers disappeared without obvious callus development. Secondary invasion by beetles and *A. thouarsianum* did not develop on smaller cankers and was limited and became inactive within a few years in larger cankers.

Among the 97 trees that showed initial SOD symptoms between 2001 and 2010, about a third were dead by 2012 (fig. 3). Most of these died within 2 years, while the remainder declined more slowly. However, more than half of all trees that developed symptoms over this period either had inactive cankers or had become asymptomatic by 2012. The cohort of trees that had early SOD symptoms at the start of this study in 2000 (n = 93), showed similar disease trajectories (fig. 3). Fewer trees from this latter cohort died within 2 years, possibly because many of the rapidly-declining trees were already dead or had late symptoms by 2000.

Phytophthora ramorum was isolated on selective media from cankers on 73 trees between 2002 and 2011. Among these confirmed SOD-positive trees, 25 percent died and 42 percent either developed inactive cankers or were no longer symptomatic by 2012. Trees with sampled or non-sampled cankers showed similar rates of symptom remission or further symptom development, indicating that sampling did not affect disease progress.

Many trees showing symptom remission were re-infected in subsequent years. In 2004, 40 trees that had previously (2000-2003) exhibited early symptoms with bleeding cankers had inactive infections or were asymptomatic. Of these, 15 (37.5 percent) developed new bleeding in 2005 or 2006. The bleeding in most of these trees came from cankers that were offset both radially and vertically from previous cankers, suggesting that most of the new bleeding arose from new cankers rather than reactivated existing cankers.

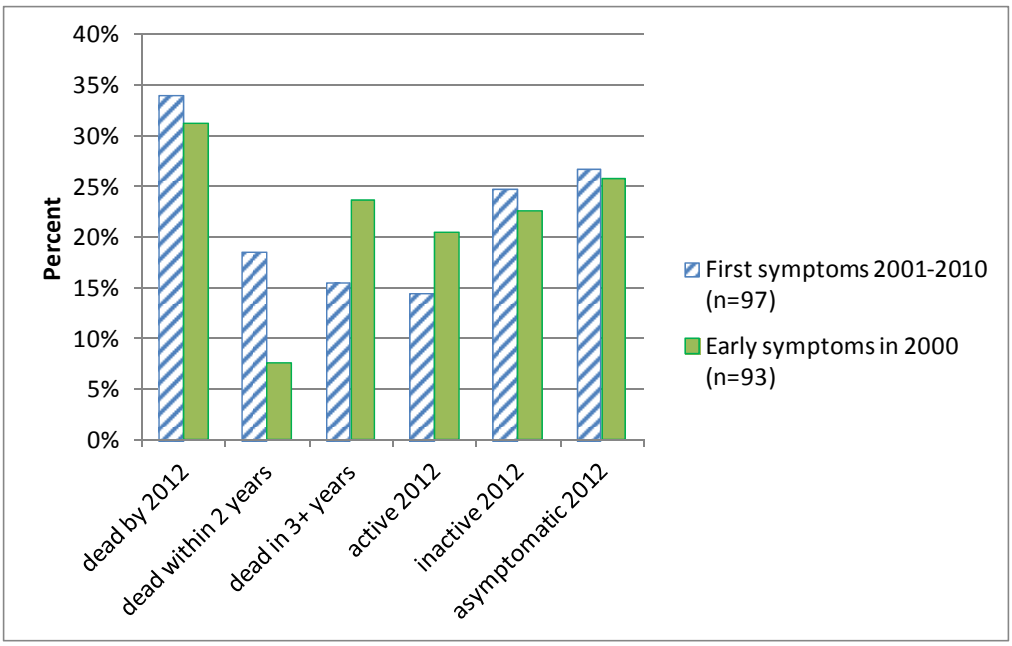

Figure 3—Disease trajectories by 2012 for cohorts of coast live oaks that developed SOD symptoms between 2001 and 2010 or had early SOD symptoms in 2000.

Factors related to disease risk

Although year to year increases in new infections were clearly associated with above-average rainfall, disease levels between locations varied greatly in a fashion that could not be explained by climate differences. In 2000, SOD incidence in coast live oak at the 10 study locations ranged from 6.2 to 52 percent; in 2012, the range was 8.9 to 56 percent. Significant differences in SOD incidence (likelihood ratio test P <0.0001) have persisted between locations over the study period even between many locations are only one to a few kilometers apart and do not differ substantially with respect to rainfall or temperature regimes. Between 2000 and 2012, the increase in disease incidence between locations ranged from 0 to 14.8 percent, again with no clear relationship to either geographic location or initial (2000) disease incidence.

We used several modeling techniques to identify variables that were related to disease development. Variables that describe both the proximity and amount of California bay located near oaks within the plots were consistently strong predictors of SOD occurrence. However, additional explanatory variables greatly improved prediction of SOD in coast live oak. High stem water potential (low water stress) and greater bark thickness were significant positive predictors of SOD symptoms. These variables were only recorded for case and control trees because measuring these variables requires extra time and effort. Among more easily observed variables that were recorded for all trees, higher density of unweathered bark fissures, greater stem diameter (strongly correlated with bark thickness), and high canopy exposure (dominant canopy position) were significant predictors of SOD symptom development.

To develop a multivariate logistic regression model, we used a binary variable indicating that SOD symptoms were present in any year between 2000 and 2012 as the outcome. Models were compared using AICc and the area under the ROC curve. The best model to date is shown in table 1. The overall misclassification rate of the

model is 23 percent and the area under the ROC curve was 0.826. The model includes two terms describing the local bay neighborhood, indicating that bay close to the trunk (2 m or less) and high bay cover within 5 m of the trunk increase SOD risk. The other two terms indicate that trees that are more dominant (higher sky exposure, often larger) and faster growing (more unweathered bark fissures) have a higher risk of SOD. Expanding bark fissures are also common sites of infection, based on field observations of recent cankers.

Table 1—Logistic regression model for SOD symptoms for any year 2000 – 2012 based on all plot trees (n=521, overall model P <0.0001)

Source	Likelihood ratio χ^2	P level	Odds ratio per regressor unit	Odds ratio per regressor range
Bay cover within 5 m (0-4 scale)	23.36	<0001	1.94	14.24
Minimum bay clearance 2 m or less	10.90	0.0010	2.67	2.67
Unweathered bark fissure rating1 (2005)	29.98	<0001	1.53	13.00
Sky exposed canopy[a] (2002)	39.18	<0001	1.53	12.79

[a]Rated using 0-6 pretransformed percentage scale

Compared with relatively healthy trees, trees declining due to other diseases only rarely developed *P. ramorum* cankers. Among 165 trees rated as in decline or that died from other causes by 2012, only 13 percent developed any SOD symptoms between 2000 and 2012. In contrast, 48 percent of 489 otherwise healthy trees developed SOD symptoms at some point over this period (difference in proportions P <0.001).

Discussion

Sudden oak death has become the primary cause of coast live oak mortality in the study plots (fig. 1). This disease is especially destructive because it preferentially affects tree that are dominant, healthy, and relatively fast growing (table 1; Swiecki and Bernhardt 2002, 2006). Over the course of this study, no predisposing host stressors have been identified or suggested from extensive field observations. Opportunistic secondary invaders, including various wood-boring beetles and decay fungi such as *A. thouarsianum* and *Phellinus gilvus*, are involved in SOD, but attack trees only after *P. ramorum* cankers have caused extensive phloem death.

The effects of SOD would be much greater if the disease epidemic were not constrained by a number of factors. Precipitation is clearly one of the strongest constraints within the study areas (fig. 2). Substantial numbers of new infections develop only in years with high rainfall and successive years of heavy precipitation are especially favorable for disease. These conditions favor the build up of inoculum on infected California bay foliage which moves to oaks mainly via splashed and windblown rain droplets (Davidson and others 2005).

The importance of nearby California bay as an inoculum source is apparent from disease models (table 1; Swiecki and Bernhardt 2002, 2008). California bay cover and density are also good predictors of SOD in disease risk models focused on the landscape scale (Kelly and Meentemeyer 2002, Meentemeyer and others 2004). For individual trees, the proximity of California bay foliage increases the likelihood that

the amount of inoculum splashed onto oak trunks is sufficient to initiate infections. More abundant California bay cover in the oak neighborhood increases the amount of inoculum that will impact an oak and also increases the size of the local reservoir for inoculum carryover during dry seasons (Davidson and others 2011). Because disease risk is highest when California bay is close to the oak, removal of bay within a few meters of susceptible oaks can greatly reduce disease risk (Swiecki and Bernhardt 2013).

Sudden oak death risk and disease progress are also strongly affected by tree characteristics (table 1; Swiecki and Bernhardt 2002, 2006). In particular, actively expanding bark fissures may be susceptible to infection due to their minimal layer of dead rhytiderm, allowing for easier infection by zoospores and sporangia. Wet conditions favor *P. ramorum* inoculum production, but also promote tree radial growth. Healthy, dominant oaks with relatively low water stress develop expanding bark fissures as the result of strong radial growth. Trees with more bark fissures also develop greater numbers of infections, which can consolidate to form larger and more severe cankers.

Based on repeated observations of hundreds of trees over a 13 year period, we now know that *P. ramorum* infections in coast live oak do not follow a single trajectory of rapid decline and mortality. That pattern, seen in less that 20 percent of symptomatic trees (fig. 3) is only one of several alternative outcomes. Slow decline over many years is about as common as rapid decline. More significantly, canker inactivation and disease remission were seen in more than half of the trees in cohorts that developed first symptoms in 2001 to 2010 or had early SOD symptoms in 2000 (fig. 3). These different patterns of disease progress were not apparent when SOD was originally described in California (Garbelotto and others 2001, Rizzo and others 2002). Understanding of these potential disease outcomes is essential for the informed management of SOD-affected forests over time (Swiecki and Bernhardt 2013).

The relatively high incidence of canker inactivation and disease remission suggests that many coast live oaks have some level of general or horizontal resistance. Such resistance may be overcome by high inoculum levels or particularly favorable environmental conditions (Erwin and Ribeiro 1996), which is consistent with the reinfection we observed in trees that had limited initial cankers. Phenolics have been identified as possible biomarkers of disease resistance in coast live oaks (McPherson and others, Biomarkers identify coast live oaks that are resistant to the invasive pathogen *Phytophthora ramorum*, these proceedings). The development of phenolics or other host defense compounds related to resistance, though ultimately under genetic control, may be strongly influenced by host stress and growing conditions, and could account for differences in susceptibility between declining and vigorous oaks.

Acknowledgements

This research was supported by cost-share grants from the USDA Forest Service, Pacific Southwest Research Station, and USDA Forest Service, State and Private Forestry Section, Forest Health Protection Program.

References

Davidson, J.M.; Patterson, H.A.; Wickland, A.C.; Fichtner, E.J.; Rizzo, D.M. 2011. **Forest type influences transmission of *Phytophthora ramorum* in California oak woodlands.** Phytopathology 101: 492–501.

Davidson, J.M.; Wickland, A.C.; Patterson, H.A.; Falk, K.R.; Rizzo, D.M. 2005. **Transmission of *Phytophthora ramorum* in mixed evergreen forest in California. Phytopathology** 95: 587–596.

Erwin, D.C.; Ribeiro, O.K. 1996. ***Phytophthora* diseases worldwide.** St. Paul, MN: American Phytopathological Society Press.

Garbelotto, M.; Svihra, P.; Rizzo, D.M. 2001. **Sudden oak death syndrome fells three oak species.** California Agriculture 55(1): 9–19.

Kelly, N.M.; Meentemeyer, R.K. 2002. **Landscape dynamics of the spread of sudden oak death.** Photogrammetric Engineering & Remote Sensing 68: 1001–1009.

Meentemeyer, R.; Rizzo, D.; Mark, W.; Lotz, E. 2004. **Mapping the risk of establishment and spread of sudden oak death in California.** Forest Ecology and Management 200: 195–214.

Rizzo D.M.; Garbelotto, M.; Davidson, J.M.; Slaughter, G.W.; Koike, S.T. 2002. ***Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California.** Plant Disease 86: 205–214.

Swiecki, T.J.; Bernhardt, E.A. 2002. **Evaluation of stem water potential and other tree and stand variables as risk factors for *Phytophthora ramorum* canker development in coast live oak.** In: Standiford, Richard B.; McCreary, Douglas; Purcell, Kathryn L., tech. coords. Proceedings of the fifth symposium on oak woodlands: oaks in California's changing landscape. Gen. Tech. Rep. PSW-GTR-184. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station: 787–798.

Swiecki, T.J.; Bernhardt, E. 2006. **Disease risk factors and disease progress in coast live oak and tanoak affected by *Phytophthora ramorum* canker (sudden oak death).** In: Frankel, Susan J.; Shea, Patrick J.; Haverty, Michael I., tech. coords. Proceedings of the sudden oak death second symposium: the state of our knowledge. Gen. Tech. Rep. PSW-GTR-196. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station: 383–411.

Swiecki, T.J.; Bernhardt, E. 2008. **Increasing distance from California bay reduces the risk and severity of *Phytophthora ramorum* canker in coast live oak.** In: Frankel, S.J.; Kliejunas, J.T.; Palmieri, K.M., tech. coords. Proceedings of the sudden oak death third science symposium. Gen. Tech. Rep. PSW-GTR-214. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station: 181–184.

Swiecki, T. J.; Bernhardt, E.A. 2013. **A reference manual for managing sudden oak death in California.** Gen. Tech. Rep. PSW-GTR-242. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. 129 p.

Swiecki, T.J.; Bernhardt, E.; Aram, K.; M.; Rizzo, D.M. 2013. **Diagnosis and management of *Phytophthora ramorum* in canyon live oak, an atypical bole canker host.** In: Frankel, S.J.; Kliejunas, J.T.; Palmieri, K.M.; Alexander, J.M., tech. coords. Proceedings of the sudden oak death fifth science symposium. Gen. Tech. Rep. PSW-GTR-243. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station: 80–83.