

Rethinking *Phytophthora*—Research Opportunities and Management¹

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Introduction

It was the second week of June, 2000, the hottest weather on record in San Francisco - hardly *Phytophthora* weather. But it was that week, at China Camp State Park, that Dave Rizzo and colleagues collected the bark samples from bleeding cankers on coast live oaks that finally moved sudden oak death (SOD) from the “cause unknown” category to “*Phytophthora* disease” (Rizzo and others 2002). Research progress has been dramatic in the last seven years. Think of the advances in *Phytophthora* genetics, capped by publication of the complete genome sequence, and its applications in diagnostics and population genetics. Think of the discovery of other *Phytophthora* species with similar life styles to *Phytophthora ramorum*, but apparently indigenous to the same forests. This was unexpected, and is stimulating a resurgence in *Phytophthora* taxonomy worldwide. Think of the substantial efforts in nursery disease research to understand the spread and survival of *P. ramorum* in this intensely manipulated environment that are leading to development of “best management practices,” and fewer and fewer infested nurseries. It would be reasonable, and comforting, to use this time at the beginning of this Sudden Oak Death Third Science Symposium to reflect on our past accomplishments. They are many. But somehow I find myself more impressed with the challenges of the future. I want to highlight some of the new and exciting work to be presented at this meeting, work that is opening new research directions, and to consider the daunting and escalating management challenges that this disease is forcing on us.

The map of the distribution of SOD in the West hasn’t changed much since the first versions went on-line in 2001. A couple of new infested counties were added in California, and new disease spots in Humboldt and Mendocino Counties and in the Big Sur are troubling, but western California hasn’t turned red. The alarm comes at a finer scale. The small patches of dead trees have multiplied, and in more and more areas the patches are now hillsides of mortality. Tanoak mortality levels exceed 80 percent on many plots, and local extinction of the species seems increasingly probable.

At the same time, it is becoming clear that coast live oak is less susceptible than tanoak; perhaps the disease will prove manageable in oak woodlands. One of the breakthroughs in research has been the realization that *P. ramorum* causes very

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different diseases on its many different hosts (Davidson and others 2003, Hansen and others 2005). Unfortunately we still have little insight into the long-term impacts of the pathogen on the many native dieback and leaf blight hosts growing in affected forests.

I have organized this presentation in three parts, two highlighting research that I find particularly exciting, and a final section challenging our disease management (in-) actions:

- Look Up!
Phytophthora epidemiology
- What's so SUDDEN about SOD?
Pathogenesis
- Fight Them on the Beaches
Disease Management

***Phytophthora* Epidemiology**

Phytophthora ramorum is an aerial *Phytophthora*. The pathogen does get into streams and the soil and persists there, and it can infect roots under some conditions at least. There is much left to discover about its behavior below ground. But in the forest especially, the action is above ground.

Phytophthora ramorum is spreading in three very different patterns in the forest: 1) local intensification of disease is driven by sporangia splashing and dripping downward from high infections; 2) the pathogen is spotting across the landscape, initiating new disease foci, probably by turbulent dispersal of sporangia in dry air; and 3) infected plants are moving very long distances in the nursery trade, threatening new forest infestations in distant parts of the country and the world. Intensive efforts to cut off the nursery pathways are having increasing success. Rain splash dispersal is well documented now in several forest types (Davidson and others 2005; Davidson, Patterson and Rizzo, in press). It is important at distances out to 10 or 20 m from a source. Turbulent dispersal is the new story for *P. ramorum*.

This pathogen is well adapted for aerial spread. Both California bay (in California) and tanoak are important hosts, supporting sporulation on leaves and twigs. Chlamydospores are formed inside infected leaves, but in the lab at least, they also form on the surfaces of leaves and twigs. Certainly they are transported as trees defoliate, and are released into the soil as leaves decompose (Fichtner and others 2007). Are chlamydospores that formed on leaf surfaces free and effective inoculum spread by rain splash? We have much to learn about chlamydospores as infection propagules. They are built to withstand harsh conditions. What triggers them to germinate?

Deciduous sporangia are the hallmark of an aerial *Phytophthora*. They form on leaves and twigs, and are readily dislodged by rain drops. Splash dispersal is an important part of SOD epidemiology (Rizzo and others 2005). But how to explain the observed dispersal across hundreds and even thousands of meters? This is well beyond the normal range of splash dispersal. Storm driven rain has been invoked, but why not turbulent dispersal? There is ample precedent in related pathogens.

Blue mold of tobacco, caused by the oomycete *Peronospora tabacina*, and late blight of potato, caused by *Phytophthora infestans*, provide well documented model systems. Both are characterized by deciduous sporangia formed on infected leaves in the canopy of the crop. The sporangia form in relatively cool, moist air at night, and are released into turbulent air currents as the atmosphere above the crop warms and dries in the daytime. Clouds of sporangia may be released from infested crops, lofted on rising turbulent air, and transported for hundreds of meters, even thousands of kilometers in the case of blue mold, before settling out of the air, or more commonly being washed out by rain (Aylor 1999, Aylor and others 1982, LaMondia and Aylor 2001, Zwankhuisen and others 1998). Infection at a great distance is a rare event, but with high inoculum loads, susceptible hosts, and favorable winds with appropriate atmospheric conditions, it happens regularly in potato and tobacco.

Is this dry air turbulent dispersal important for SOD? The evidence is yet incomplete, but it seems likely: 1) Tanoak trees are infected in the upper canopy, and deciduous sporangia are formed (Rizzo and others 2005); 2) The pattern of new infections in the Oregon eradication area mirrors a classical turbulent transport dispersal gradient (Gregory 1968) (Fig. 1); and 3) Microsatellite genotyping shows that new infections (up to 4 km distant) in Oregon are coming from inside the infested area, not from outside (Prospero and others 2007). When might turbulent dispersal occur? Let us suppose it takes two days with very high humidity and temperatures 15°C for sporangia to form on leaves (Englander and others 2006), followed by a short period of

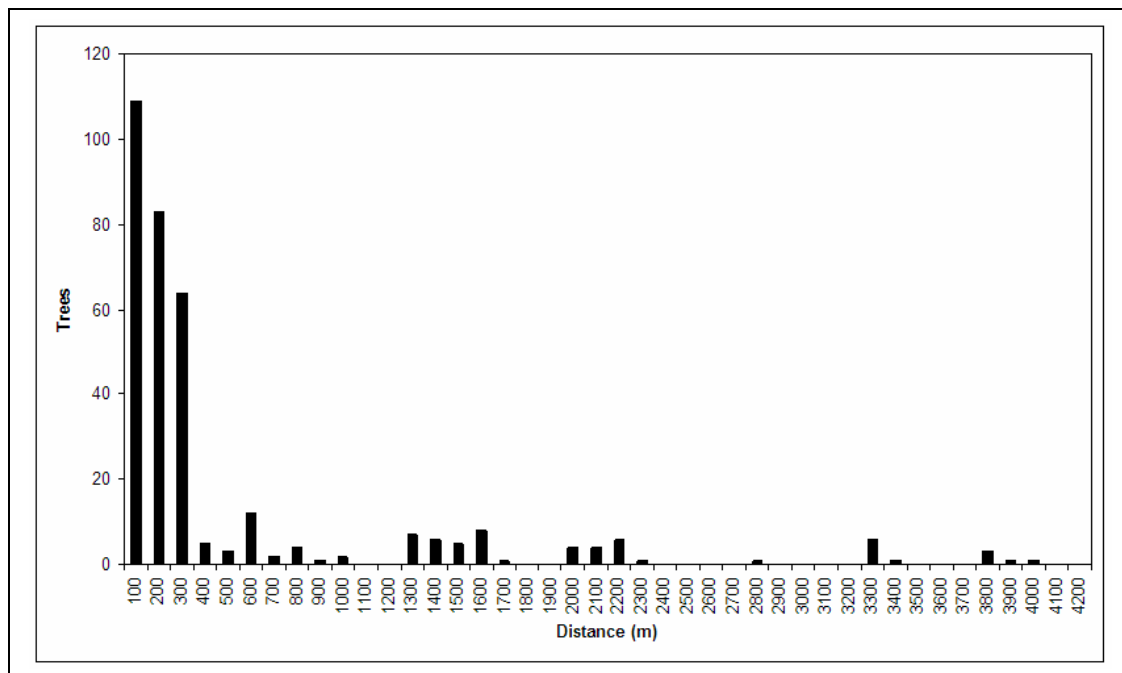


Figure 1—Distance between tanoak trees killed by *P. ramorum* and the nearest tree killed by the pathogen in a previous year.

warming and drying to snap the sporangia off of their stalks and establish rising air currents. Add a gentle breeze and they are on their way. Gravity, or cooling air, or rain will bring them down. Yes, sporangia are vulnerable to drying and UV light, but the potato and tobacco pathogens, at least, survive even rather harsh conditions for an hour or so (Bashi and Aylor 1983, Englander and others 2006, Mizubuti and others 2000). I imagine a warm wet period in Curry County in the spring, with new, vulnerable growth on the trees. With clearing weather, rain is replaced overnight by dew and fog. The fog breaks up as temperatures rise and the day progresses, lofting the sporangia. Cooling air settles downslope in the evening and the fog forms again, providing moisture now for germination of sporangia on tender new leaves.

***Phytophthora ramorum* Pathogenesis**

What is so sudden about “sudden oak death?” We know now that it takes at least a year from initial infection of a tree to death, even in the very susceptible tanoak (McPherson and others 2005). Death isn’t really a sudden process in trees, yet the entire crown often turns red all at once. A tree is green one season and red the next. The culmination of some of the earliest SOD work, and the initial results of new research directions are helping us understand just how, and why, infected trees are killed by this disease.

Oak bark beetles were among the first suspects as causal agents of SOD. We now know that they are secondary invaders, attracted to and breeding in trees already made attractive by *Phytophthora ramorum* (McPherson and others, this Proceedings). The pathogen can and does kill oaks without the aid of beetles, but it usually doesn’t get the chance. Oak bark beetles are ubiquitous and often numerous, and find diseased trees quickly. Their galleries, and the staining fungi that accompany them, quickly kill the inner bark and living cells in the sapwood, further blocking water movement in the trunk, and leading to death of the crown. Trees attacked by bark beetles after *P. ramorum* infection die more quickly than trees infected by the pathogen alone (McPherson and others 2005).

Oak ambrosia beetles also contribute to the “sudden” appearance of the disease. These beetles burrow into the sapwood of trees to excavate their egg galleries. Like the bark beetles they are secondary attackers, and like the bark beetles they carry pathogenic fungi with them that hasten death of cells and decay the wood of oaks and tanoaks. Tanoak appears to not form heartwood. Sapwood, and ambrosia beetle galleries, extend right through the tree. One aspect of “sudden” in SOD is sudden breakage and collapse of the tree. This results in large part from the galleries and associated wood decay of the ambrosia beetles.

Back to the primary agent of SOD, *P. ramorum*. How does it kill the tree? The new part of the story is shifting the focus from the iconic bleeding cankers in the bark to behavior of the pathogen in the sapwood beneath the bark cankers.

The classical view of *Phytophthora* in trees focuses on bark cankers, with trees girdled by necrosis of the inner bark, the phloem and the cambium. There have been a few observations of other *Phytophthora* species in sapwood of their various hosts (Davison and others 1994, Oh and Hansen 2007), but they attracted little attention. It was noted early on that the sapwood behind *P. ramorum* bark cankers was sometimes discolored. Both Dave Rizzo and I isolated the pathogen from sapwood on occasion but didn't follow up. That has now changed. Two research groups, led by Anna Brown in England (Brown and Brasier 2007), and Jennifer Parke in this country (Parke and others 2007), have demonstrated that sapwood colonization is a regular and important feature of *Phytophthora* pathogenesis in several tree/*Phytophthora* combinations, including tanoak/*P. ramorum*. The British are suggesting that not only does *P. ramorum* survive from year to year in the sapwood, but that it also spreads up and down the tree in the xylem, creating new bark cankers, and bleed spots, from the inside out.

Work in Oregon has moved on to understanding the impact of xylem colonization on pathogenesis. *P. ramorum* was monitored in wood by isolation, microscopy, and PCR. Hyphae were seen in various cell types, but especially in xylem vessels. Chlamydospores were also present in vessels, and in many cases tyloses had ballooned through the vessel bordered pits from adjoining parenchyma cells. The net results were visible obstructions within the xylem vessels, and impeded water flow (Parke and others 2007).

Sap flow was monitored in green, infected trees and in uninfected trees. There was a significant reduction in xylem water transport in infected trees, before crown symptoms were evident. What other impacts might *P. ramorum* hyphae in the xylem have on host physiology?

Dan Manter, United States Department of Agriculture (USDA)-Agricultural Research Service (ARS) in Fort Collins, Colorado, has been working with the elicitors formed by *P. ramorum* (Manter and others this Proceedings). Elicitors are a class of low molecular weight proteins produced by *Phytophthora* species. They function as sterol transport proteins, damaging host cell membranes and carrying the released sterol molecules back to the mycelium (Bonnet and others 1996, Mikes and others 1998). *Phytophthoras* require exogenous sterols for reproduction. Each *Phytophthora* species produces its own specific elicitors. Dan has isolated the *P. ramorum* elicitors, and compared their effects on host tissues with the effects of the intact pathogen (Manter and others 2007). Both *P. ramorum* infection, and elicitor uptake from purified culture filtrates, trigger early reductions in carbon assimilation, stomatal conductance, and water transport. Elicitors are evidently key players in pathogenesis (Ricci 1997).

Managing *P. ramorum* in Western Forests

“Fight them on the beaches, or let the new order begin”
Professor Hal Mooney, Stanford University

Dr. Mooney's pronouncement was directed at the worldwide ecological threat from invasive organisms in general, but it encompasses the range of disease management situations forest pathologists face with *P. ramorum* very well. The fighting is fierce and increasingly successful in the nurseries, with the quarantine and eradication

regulations designed to keep SOD from spreading to new areas around the country and the world. In Oregon, the forest epidemic is being confronted acre by acre, with a local eradication campaign designed to halt the further spread on this particular front. The situation in California is much more complex, however, with the pathogen well established in many areas and still spreading in others.

In 2000, within a few months of identification of a new *Phytophthora* as cause of SOD, Oregon had begun early detection surveys, and in 2001, within weeks of locating the initial SOD infestations in the state, the eradication effort had begun. Despite early detection and prompt action, six years later we have not yet succeeded in eradicating the pathogen from its Oregon beachhead (Kanasie this Proceedings). The pathogen is successfully neutralized on treated sites, but it continues to jump to new areas ahead of our eradication efforts. The net effect is a more or less stable infestation; we have, at least temporarily, contained the pathogen by preventing a dramatic increase in inoculum. The relative success of the Oregon effort is evident when compared to the explosive spread of SOD in Humboldt County, California (Fig. 2). The disease was first detected at about the same time, across roughly the same area in the two counties.

California lacks an articulated overall strategy for ramorum management. Without aggressive action, there is little to do except watch the “new order,” the aftermath forest without tanoak, develop. The “no action alternative” future is increasingly clear. We don’t need to guess any longer about the possible ecological impacts of *P. ramorum* on California forests. Tanoak is rapidly disappearing from expanding areas of Marin, Sonoma, Santa Cruz, Humboldt and Mendocino counties, and the Big

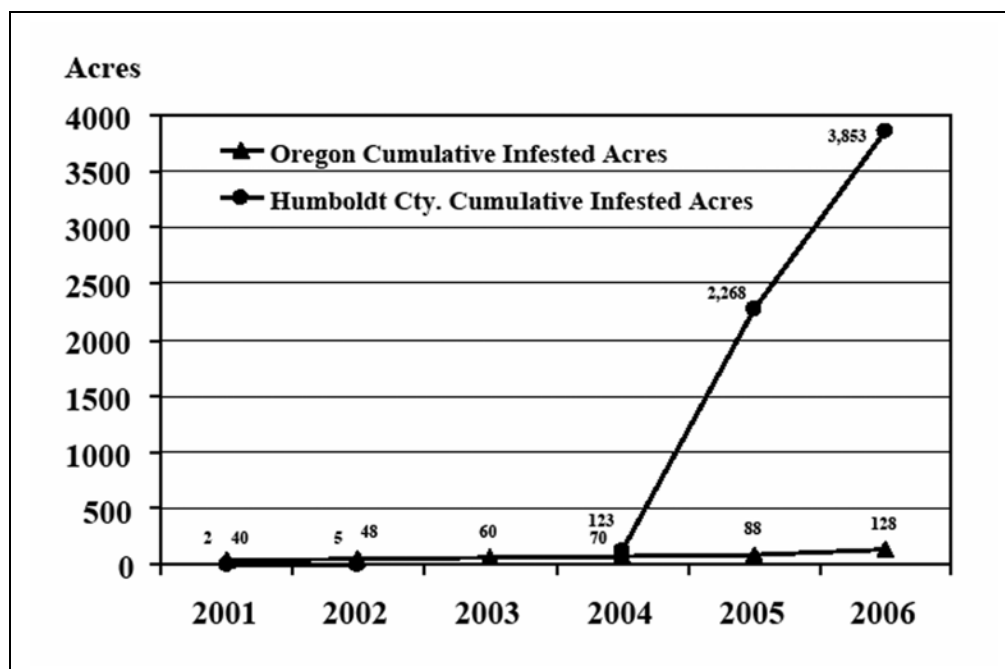


Figure 2—Increase in tanoak forest area affected by *P. ramorum* from 2001-2006, in Humboldt county, California and Curry county, Oregon.

Sur region of Monterey County. The epidemic has created a valuable ecological laboratory for the study of the impacts of invasive disturbance on ecosystem function, and several labs are taking advantage of the opportunity (Rizzo and others 2005, Maloney and others 2005). Exciting work is being done. There is still a lot of tanoak left in other parts of the state, however, and good opportunities remain to try to stop the further spread of the pathogen, especially to the north and east.

One obstacle to a concerted SOD control program is ambivalence about the importance of tanoak in western forests. Tanoak has essentially a negative economic value in the timber industry. It is most often viewed as an aggressive weed, competing with much more valuable conifers for growing space. Its ecological values need to be articulated more clearly, including its important roles as a mast producer (important to indigenous peoples as well as to wildlife), and as an early colonizer and stabilizer of disturbed sites.

It is also important to remember the economic impacts of regulatory actions triggered by further spread of SOD. These direct losses and indirect costs are already documented for the horticultural nursery industry. Douglas-fir is another host for *P. ramorum*, unlikely to be seriously impacted in commercial forests, but still subject to unpredictable international quarantine regulation.

It comes down to a simple question: “How serious are we about controlling SOD?” We have learned enough in the last six years to make that a manageable question, worthy of site specific consideration. It need not be all or nothing. The question should be answered separately for coast live oak forests, where managing California bay and judicious use of phosphonate may prove sufficient to save large numbers of trees in critical landscapes. The western Sierra Nevada in California is considered at lower risk to SOD because of climate. That suggests that the disease won’t spread as fast there, and that aggressive eradication efforts have a better chance of success. Are we surveying regularly for early detection, and most importantly, are we administratively ready for prompt action when the pathogen is confirmed in the Sierra? Who will do what, and who is going to pay for it? The same questions of responsibility and readiness hold for the high risk forests of the eastern U.S.

Are we serious enough about stopping the northward spread of SOD to try a landscape level approach? The answer has been “no,” in California, but that can change. Why aren’t we using aerial applications of phosphonate to stop the advance of this disease? They do in Australia, against a related pathogen (Hardy and others 2001). A reasonable answer would be “because we don’t know if it will work on *P. ramorum* and tanoak.” But then why aren’t we trying to find out if it will work? What about host-free barriers across the landscape? Dramatic? Certainly, and not to be undertaken lightly, but certainly worthy of careful consideration and reasoned debate.

Finally, I want to highlight another aspect of the SOD phenomenon. New models of research support and collaboration have been generated that should continue to produce benefits for years to come. The several branches of the Washington Office of the USDA-Forest Service have been central to the program. They allocated money to get things started, and worked with California politicians and Congress to generate substantial and continuing support. The Pacific Southwest Research Station, and Pacific Southwest Region of the Forest Service have been creative and unstinting

coordinators of an ever expanding research and disease management program. My California university colleagues especially have leveraged seed money from the Forest Service into National Competitive Grants from the National Science Foundation (NSF) and other programs, the Joint Genome Initiative, and Private Foundations.

New patterns of cooperation and collaboration have been forged, between institutions and agencies, between states, and internationally. The participation of our European colleagues has been invaluable in both the science and the regulation of *P. ramorum*. The California Oak Mortality Task Force is unique and invaluable. In Oregon, the convergence of skills and commitment and camaraderie in our small team is the highlight of my career. Thank you all.

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