Phytophthora ramorum and Sudden Oak Death in California: II. Transmission and Survival[1]

Jennifer M. Davidson,[2] David M. Rizzo,[3] Matteo Garbelotto,[4] Steven Tjosvold,[5] and Garey W. Slaughter[3]

Abstract

The newly discovered *Phytophthora ramorum* canker disease of oak (Sudden Oak Death Syndrome) threatens millions of acres of California woodlands where coast live oak (*Quercus agrifolia*), tanoak (*Lithocarpus densiflorus*), or black oak (*Quercus kelloggii*) are dominant species. An important step in controlling this disease involves understanding how it is spread. The presence of diseased oaks at all elevations on hillsides and the above-ground nature of the disease suggest wind-blown rain or rain splash as a common mechanism for movement of spores. Although viable spores have yet to be found on infected oak tissue, other hosts may serve as sources of rain-dispersed inoculum. In the laboratory, abundant sporangia form on moistened leaves of infected bay (*Umbellularia californica*) and *Rhododendron* spp. within 72 hours. These sporangia break off and easily disperse in water. Chlamydospores were also observed on the surface of moistened bay leaves. Consistent with these results, *P. ramorum* has been recovered from rain, soil, litter, and stream water from woodlands with infected oak and bay trees. Spores of *P. ramorum* do not survive drying, but in moist conditions can survive for at least one month.

Introduction

The newly described *Phytophthora ramorum* canker disease of oak (Sudden Oak Death Syndrome) threatens millions of acres of California woodlands where coast live oak (*Quercus agrifolia*), tanoak (*Lithocarpus densiflorus*), or black oak (*Quercus kelloggii*) are dominant species (Garbelotto and others 2001, McPherson and others 2000). An important step in controlling this disease involves understanding how it is spread, both on a small scale between oak trees in an infected area, and over a larger scale from infected to uninfected woodlands. Transmission consists of spore production, movement of spores, and infection of a new individual. Knowledge of the necessary conditions for each of these steps may help us establish barriers to the

[1]An abbreviated version of this paper was presented at the Fifth Symposium on Oak Woodlands: Oaks in California's Changing Landscape, October 22-25, 2001, San Diego, California.

[2] Research Plant Pathologist, Pacific Southwest Research Station, USDA Forest Service, P.O. Box 245, Berkeley, CA 94701 (e-mail: jmdavidson@ucdavis.edu)

[3] Associate Professor and Staff Research Associate, respectively, Department of Plant Pathology, One Shields Ave., University of California, Davis, CA 95616

[4] Assistant Professor, Department of Environmental Science, Policy and Management, Ecosystem Science Division, 151 Hilgard Hall, University of California, Berkeley, CA 94720

[5] Farm Advisor, University of California Cooperative Extension, 1432 Freedom Blvd, Watsonville, CA 95076

spread of infection within a single park or between geographically separated host plant ranges such as the Coastal mountains and the Sierra Nevada of California.

Phytophthora species generally reproduce through both dormant (resting) spores, as well as rapidly produced fruiting bodies (sporangia) that contain swimming spores (zoospores). Dormant spores may either be sexual (oospores) or asexual (chlamydospores) (Erwin and Ribeiro 1996). The production of sporangia generally requires very high humidity, and therefore, will be strongly affected by seasonal temperatures and rainfall (Duniway 1983). Survival of all spore types will also be affected by temperature and moisture (Duniway 1983). In addition, production and viability of spores may differ on each host species.

Once produced on or within the host plant, spores may spread via rain, soil, stream water, or movement of the infected plant itself. Movement of plant material may include infected nursery stock, firewood, or timber. Transport of soil containing propagules may occur on shoes of hikers, tires of trucks and mountain bikes, or the feet of animals. Movement of spores in stream water may allow for long-distance transport, depending on viability of spores in water, and the potential of spores to move from stream water to the susceptible parts of host plants (Hansen and others 2000). Rain splash has been shown to move *Phytophthora* spores of other species over several meters (Ristaino and Gumpertz 2000), making this a potentially effective means of local transport, especially for spores requiring moisture for production and survival.

The mode of transmission for *P. ramorum* among California oaks is complicated by the presence of many other host plant species in the forest. Currently, the host list includes 10 non-oak plant species in five plant families (Rizzo and others 2002). These other hosts may serves as sources of inoculum and act as infectious links among oak trees. In addition, nursery stock of host plant species, such as rhododendron, can serve as vectors of disease when moved for landscaping.

In this paper, we provide data on the types of spores produced by *P. ramorum* under laboratory and natural host plant conditions. We also document the seasonality of spore production in recovery from rainwater, soil, and stream water. Finally, we use survival of zoospores and chlamydospores in the laboratory in water, and on dry and moist filter paper to simulate viability of spores in stream water, and under moisture extremes on solid substrates, such as litter or soil.

Methods

Production of Spores

A total of 119 isolates of *P. ramorum* were obtained from six host species (coast live oak, black oak, shreve oak (*Quercus parvula* var. *shrevei*), tanoak, bay, and ornamental rhododendron spp.) in 36 locations over an area approximately 300 km long from Monterey to Mendocino County, and ranging up to 65 km inland (Rizzo and others, in press). These isolates were grown on pimaricin-ampicillin-rifampicin-PCNB agar (PARP) and Corn Meal Agar (CMA) in the laboratory and examined for spore production. They were also cultured on V8 medium and V8 agar plugs were put in soil water extract at 18-23 °C for 24 hours to examine sporangia production. (For methods, see Rizzo and others, in press.)

Production of spores were also monitored on pieces of naturally infected host tissue collected from forest and nursery sites within the 300 km host range. Eight

excised coast live oak cankers, 9 infected bay leaves, and 15 infected nursery rhododendron leaves were placed in moist chambers at 18-23 °C for 72 hours and monitored for spore production. In addition, the bleeding sap was collected when possible from 13 coast live oak at weekly intervals from April 22 to July 16, 2001, and plated on PARP medium to test for the presence of viable pathogens.

Movement of Spores

During the isolation of *P. ramorum* from 93 oak and tanoak trees, field observations of the position of cankers on trees and the location of infected trees with respect to topography were used to gain insight into the mechanisms of spore movement, whether above ground, moved in soil, or carried by rain runoff or streams. Location of infection on 20 bay trees and 6 rhododendron bushes also was noted. Recovery of spores was attempted from rainwater, soil, litter, and stream water in a time series spanning the winter rains and the drying summer months.

Rainwater

Raintraps were used to collect rainwater in a coast live oak woodland at Fairfield Osborn Preserve in Sonoma Co. Both coast live oak and bay trees were infected with *P. ramorum* at this site. Raintraps consisted of a 165 cm by 75 cm vinyl sheet stretched over a pvc frame and folded into a funnel with a 4 liter collecting jar at the bottom. Traps were set up on February 2, 2001 and used to collect rainfall through winter, and for one unusually late rain on June 27-28. Two traps were placed 0.5 m in front of cankers on each of 7 oak trees, for a total of 14 traps. Previous isolations confirmed that the cankers were caused by *P. ramorum* infection. Two "distant" traps were placed at a distance of 5 m from all infected oak trees. Four additional "distant" traps were installed on March 15, 2001. Although separated from infected oaks, "distant" traps still were under the forest canopy, which consisted mainly of bay trees. Rain water was collected at approximately 2-day intervals during a storm event. Rainwater was stored overnight at 4-5 °C to allow spores to settle. One liter of water was suctioned off of the bottom of containers and filtered through a Millipore 3 μ cellulose esterase filter to capture all spore types. The filter was then cut into strips and placed filtrate side down on selective medium plates. After 7 days, strips were removed, and colonies of *P. ramorum* were counted.

Soil

Beginning in March, 2001, soil and litter were collected on a monthly basis from around the base of 15 diseased oak trees at the Fairfield Osborn Preserve to test for the presence of *P. ramorum.* Previous isolations confirmed that all trees were infected by *P. ramorum*. At the base of each tree on the infected side, soil was collected in zip-lock bags from three separate spots and pooled to equal 500 g. Litter was collected at three spots to fill one-fourth the volume of a gallon zip-lock bag. A green d'anjou pear was pressed into the soil or litter in each sample so that one-third of the pear was immersed, and diH20 was added to the sample until one-half of the pear was immersed in water. Samples were allowed to sit for 6 days at 18-23 °C. Pears were then removed, washed, and monitored for signs of *Phytophthora* lesions. Tissue from likely lesions was plated on PARP selective medium to verify *P. ramorum* presence.

Stream Water

Water was collected from Bean Creek, Santa Cruz Co., on a bi-monthly basis from April through June 2001 in a forested area with infected coast live oak and tanoak trees. Sampling ended in June because the stream dried up. For each sample, 8 liters of stream water were stored in plastic bins at 18-23 °C. Two d'anjou pears were added to each bin so that pears were halfway immersed in stream water. After 5 days, pears were removed and *P. ramorum* infection was assessed as for soil baiting (see above).

Survival of Spores

To assess survival of spores under various moisture regimes, suspensions of zoospores and chlamydospores of *P. ramorum* were each added to water, moist filter papers, or dry filter papers (Fisherbrand P4 4.25 cm) and monitored for viability for one month. The suspensions for both types of spores consisted of a pool of spores from fifteen isolates. Approximately 200 spores in 100 ul of suspension were added to either (1) 100 ul of diH20; (2) a filter moistened to saturation with 100 ul diH20; or (3) a dry filter. The moistened filters were kept in closed screw-cap tubes. An additional 150 ul dH20 was added to the moist filter at two week intervals to maintain saturation. The dry filters were allowed to dry completely at room temperature (23 °C, 30 percent rh, 30 minutes) and placed in a crisper. All treatments were stored at 15 °C. Five replicates of each treatment were plated on selective medium at 0, 3, 7, 15, and 30 days for chlamydospores, and 0, 7, 15, and 30 days for zoospores. Colonies were counted at 36 and 72 hours to assess viable spores.

Results

Production of Spores

In laboratory culture, all 119 isolates of *P. ramorum* produced large numbers of chlamydospores, the asexual resting spores. In addition, all isolates produced large numbers of sporangia within 24 hours when grown on V8 agar plugs in soil water. These sporangia were highly deciduous at 20 °C, meaning that they easily detached from the hyphae for dispersal. These detached sporangia were observed to either germinate directly or to release zoospores. Oospores, the sexual resting spores, were not observed on either medium for any of the 119 isolates.

Spore production was observed on tissue of some host species. Sporangia were present on the surface of 3 of 9 infected bay leaves and all 15 rhododendron leaves within 72 hours. Chlamydospores were also observed on the surface of one bay leaf. No spores were observed on the surface of oak bark cankers. Sporangia were observed microscopically in the bleeding sap of one oak tree. However, none of the 81 plating attempts of bleeding sap from the 13 trees resulted in colony formation.

Movement of Spores

Field Observations

Observations suggest that spores of *P. ramorum* were aerially dispersed. Of the 93 oak and tanoak trees from which *P. ramorum* was isolated, none had cankers

below the root crown. Cankers on one tanoak were as high as 20 m. Of the six rhododendrons sampled, all infection was in the leaves or stems. Of 20 bays sampled, all successful isolation was from leaves, although lesions were present on some terminal twigs. Infections on oaks were observed from sea level to 800 m and at all elevational positions on hillsides ranging from valleys to ridgetops. Infection did not strictly follow water courses.

Raintraps

Rainwater contained viable spores of *P. ramorum (fig. 1)*. At some point during the sampling period, *P. ramorum* was recovered from rain captured in traps placed at all seven of the coast live oaks. This rainwater was a combination of throughfall rain, drip from the canopy, and splash from the trunk. Propagule counts were low, but present in rainwater from these traps at each of the collection periods except for the brief summer storm in late June (0.5 inches rainfall). Spore counts were variable from tree to tree, and ranged from 0.25-7 spores per liter in positive samples from traps placed at the oak trees. Of the six "distant" traps placed under the forest canopy but 5 m away from infected oak trees, two were positive for *P. ramorum* during the sampling period.

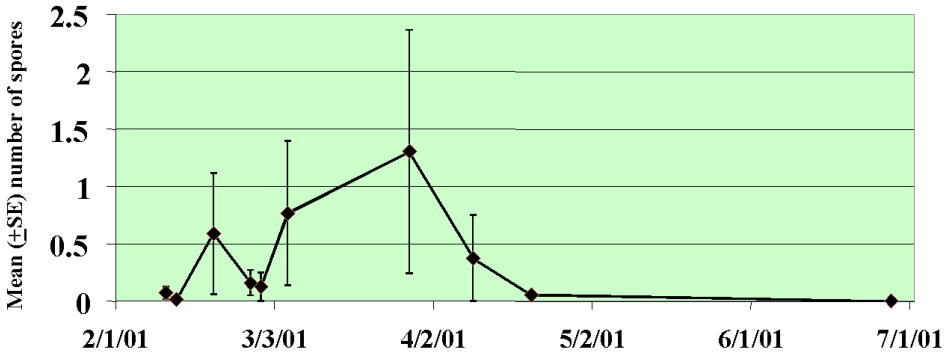

Figure 1 Mean number of *P. ramorum* spores per liter of rainwater (± standard error) for storms occurring from February through June, 2001 at the Fairfield Osborn Preserve, Sonoma Co. Means were based on the average from two collecting traps at each of seven coast live oak trees.

Soil Baiting

Soil and litter also contained viable propagules of *P. ramorum*. During March, 3 of 15 soil samples and 1 of 15 litter samples tested positive. During April, no soil samples tested positive, and only 1 of 15 litter samples tested positive. During May, no litter samples tested positive, and only 1 of 15 soil samples tested positive. The positive May sample was from the same tree as the positive April sample. In June and July, all samples were negative.

Stream Baiting

The April sample from Bean Creek was positive for *P. ramorum*. Samples from May and June were all negative.

Survival of Spores

A portion of both the zoospores and chlamydospores survived in the water and moist filter treatments *(fig. 2)*. Both types of spores were killed by the drying process at 30 percent rh at room temperature in the "dry filter" treatment (data not shown). Chlamydospores, thick-walled resting spores, survived better than zoospores in both the water and moist filter treatments. Zoospore survival averaged less than 20 percent at the end of the 30-day period while chlamydospore survival at 30 days in water and on moist filters still averaged 75 percent and 41 percent of starting values, respectively.

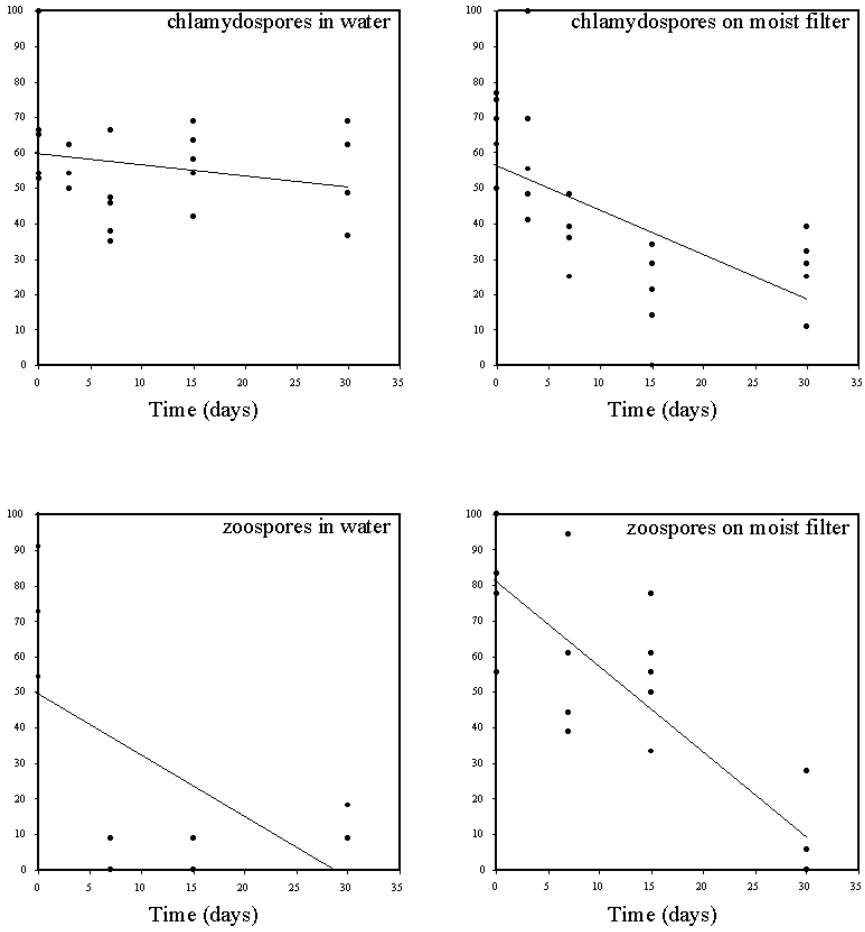

Figure 2 Survival over time of *P. ramorum* chlamydospores and zoospores in water and on moist filter paper at 15 ¡C. Each datum represents the viable spore count (scored by germination) for one of five treatment replicates plated to agar at a given time point. Spore count data is scaled as a percent of the highest spore count at time (0) to facilitate comparison among graphs.

Discussion

Current data suggest that *P. ramorum*, the causal agent of "sudden oak death," may have been introduced to northern California approximately a decade ago (Garbelotto and others 2001, Garbelotto and others 2002, McPherson and others 2000). Today, the range of the pathogen covers over 600 km, from Big Sur into southern Oregon. This rate of spread surpasses that of Chestnut Blight in the early part of the 20th century (Anagnostakis 1987). Our data on transmission and survival of *P. ramorum* provide preliminary insight into the mechanisms underlying this remarkable spread.

The rapid production of spores on foliar hosts, such as bay and rhododendron, may be key to the spread of *P. ramorum* within a locality, and perhaps between different geographic locations. *P. ramorum* produced sporangia on moistened leaves of both bay and rhododendron, the two foliar hosts tested. Chlamydospores were also noted on infected bay leaves. In addition, we have observed chlamydospores on infected rhododendron leaves in moist chambers for other experiments.

While rhododendrons are not major components of most California forests, bay trees are dominant species in many coast live oak forest types. Consistent with laboratory results on spore production, evidence from the field suggests that inoculum from bay trees may be very important in vectoring *P. ramorum* to oaks. Swiecki and Bernhardt (2001) found a significant association between infected oaks and the presence of bay trees. Rainwater traps in this study placed 5 m from infected oaks contained viable spores that may have come from overstory infected bay trees. Furthermore, we often observe an increase in *Phytophthora*-like leaf spots on bays growing within oak infection centers. To further investigate the importance of bay as an inoculum source, studies are underway to determine temperature requirements for sporangia production on bay leaves, the viability of *P. ramorum* in leaves attached to trees or fallen in litter, and the distance spores can travel from bay leaves.

The negative results of spore production on oak bark cankers or in bleeding sap require further investigation. Rapid contamination of oak cankers by fungal species may have prevented sporulation of *P. ramorum*. In addition, the seasonal state of the bark may not have been conducive to spore production at the time of the test (Brasier and Kirk 2001). However, successful amplification of *P. ramorum* DNA from bleeding sap (Garbelotto and others 2002), suggests the presence of propagules and underscores the need for additional isolation attempts from sap.

Viable spores of *P. ramorum* were shown to be carried in rainwater, soil, litter, and stream water. The presence of diseased oaks at all elevations on hillsides and the above-ground nature of the disease suggest wind-blown rain or rain splash as a common mechanism for movement of spores. The abundance of viable propagules exhibited a distinct seasonality, peaking in March and early April of 2001, a low rainfall year. Production of sporangia by other *Phytophthora* species depends on high moisture levels (Duniway 1983). Hence, the presence of moisture from rain on infected plant tissue is probably necessary for production of spores that eventually end up in soil, litter, or stream water. Generally increasing levels of inoculum in rainwater in early April, and the lack of spores in the isolated, 2-day June rain, may suggest that a time of prolonged moisture is needed for inoculum buildup. Warming temperatures in early April during the period of consistent rainfall may also have contributed to the peak in spore production. Failure to recover *P. ramorum* from soil and litter in the summer months may indicate that seasonal drying is sufficient to reduce viability of spores in these substrates.

Survival of *P. ramorum* spores also depended on moisture levels. In laboratory tests, both chlamydospores and zoospores placed in suspension on filter paper were killed by drying for one-half hour at 30 percent relative humidity. However, with moist conditions, zoospores and chlamydospores of *P. ramorum* can survive for at least a month, and this study suggests that chlamydospores probably survive much longer. Because moisture loss appears to be one way to kill spores of *P. ramorum*, we are initiating studies to determine survival times for spores under a range of humidity levels. Pairing laboratory data on moisture requirements for spore survival with climate data from forests may help us predict how long spores are present in litter and soil after rains cease, and hence, when closure of areas to the public or logging may be warranted.

Given the ability of *P. ramorum* to produce spores on foliar hosts which can then be carried in wind-blown rain, it is understandable how *P. ramorum* could readily spread among oaks within a given location. It is harder to explain long-distance jumps between known sites with oak disease: What process vectored *P. ramorum* between oak trees in Cazadero and Boonville, Boonville and Brookings, Oregon, or sites on opposite sides of the Napa Valley? Aerial dispersal of spores in wind without rain can move spores up to a kilometer (Ristaino and Gumpertz 2000). However, only two of the 60 species of *Phytophthora* are known to have this kind of dispersal (Duniway 1983). None-the-less, given the range data on oaks, we are initiating wind tunnel studies to test for dispersal of *P. ramorum* sporangia by air without rain. In addition, anthropomorphic spread of infection cannot be ruled out. Ultimately, it also remains a primary research priority to investigate forested corridors between oak disease sites for the presence of infected foliar hosts serving as infection pathways.

References

Anagnostakis, S.L. 1987. **Chestnut blight: the classical problem of an introduced pathogen.** Mycologia 79: 23-37.

Brasier, C.M.; Kirk, S.A. 2001. **Comparative aggressiveness of standard and variant hybrid alder phytophthoras, *Phytophthora cambivora* and *Phytophthora* species on bark of *Alnus*, *Quercus* and other woody hosts.** Plant Pathology 50: 218-229.

Duniway, J. M. 1983. **Role of physical factors in the development of *Phytophthora* diseases.** In: Erwin, D.C.; Bartnicki-Garci, S.; Tsao, P.H., editors. Phytophthora: its biology, taxonomy, ecology and pathology. St. Paul, MN: The American Phytopathological Society; 175-188.

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

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

Garbelotto, M.; Rizzo, David M.; Hayden, Katie; Meija-Chang, Monica; Davidson, Jennifer M.; Tjosvold, Steven. 2002. ***Phytophthora ramorum* and sudden oak death in California: III. preliminary studies in pathogen genetics.** In: Standiford, Richard, B.; McCreary, Douglas; Purcell, Kathryn L., technical coordinators. Proceedings of the fifth symposium on oak woodlands: oak woodlands in California's changing landscape. 2001 October 22-25; San Diego, CA. Gen. Tech. Rep. PSW-GTR-184. Albany, CA: Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture [this volume].

Hansen, E.M.; Goneen, D.J.; Jules, E.S.; Ullian, B. 2000. **Managing Port-Orford Cedar and the introduced pathogen *Phytophthora lateralis*.** Plant Disease 84: 4-12.

McPherson, B.A.; Wood, D.L.; Storer, A.J.; Svirha, P.; Rizzo, D.M.; Kelly, N.M.; Standiford, R.B. 2000. **Oak mortality syndrome: sudden death of oaks and tanoaks.** California Department of Forestry. Tree Note No. 26.

Ristaino, J.B.; Gumpertz, M.C. 2000. **New frontiers in the study of dispersal and spatial analysis of epidemics caused by species in the genus *Phytophthora*.** Annual Review of Phytopathology 38: 541-76.

Rizzo, D.M.; Garbelotto, M.; Davidson, J.M.; Slaughter, G.W.; Koike, S.T. 2002. ***Phytophthora ramorum* and sudden oak death in California: I. host relationships.** In: Standiford, Richard, B.; McCreary, Douglas; Purcell, Kathryn L., technical coordinators. Proceedings of the fifth symposium on oak woodlands: oak woodlands in California's changing landscape. 2001 October 22-25; San Diego, CA. Gen. Tech. Rep. PSW-GTR-184. Albany, CA: Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture [this volume].

Rizzo, D.M.; Garbelotto, M.; Davidson, J.M.; Slaughter, G.W.; Koike, S. [In press]. **A new *Phytophthora* canker disease associated with extensive mortality of *Lithocarpus* and *Quercus* in California.** Plant Disease.

Swiecki, T.; Bernhardt, E. 2001. **Evaluation of stem water potential and other tree and stand variables as risk factors in *Phytophthora* canker development in coast live oak and tanoak.** Unpublished report to the USDA Forest Service and supplied by author.