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Evidence for aerial transmission of *Phytophthora ramorum* among *Quercus* and *Lithocarpus* in California woodlands

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Abstract. The newly discovered *Phytophthora ramorum* canker disease of oak (Sudden Oak Death Syndrome) threatens millions of acres of California woodlands where *Quercus agrifolia* (coast live oak), *Lithocarpus densiflorus* (tanoak), or *Quercus kelloggii* (black oak) are dominant species. An important step in controlling this disease involves understanding how it is spread. We provide evidence for an aerial pathway of transmission for *P. ramorum*. The presence of diseased oaks at all elevations on hillsides and the above-ground nature of the disease indicate an aerial component to the movement of spores. Although viable spores have yet to be found on infected oak tissue, foliar hosts may serve as sources of inoculum that are produced and aerially dispersed in rain. In the laboratory, abundant sporangia form on moistened leaves of infected *Umbellularia californica* (bay) and *Rhododendron* spp. within 72 hours. These sporangia are highly caducous and easily disperse in water. Chlamydospores were also observed on the surface of moistened *U. californica* and *Rhododendron* leaves. Consistent with these results, *P. ramorum* has been recovered from rainwater collected from woodlands with infected oak and bay trees. *P. ramorum* has also been found in soil, litter, and streamwater. Laboratory experiments suggest that spores of *P. ramorum* that land on oak bark could survive in moist conditions for at least one month. In addition, *P. ramorum* can survive in living moistened *U. californica* leaves throughout the summer months. In a field inoculation trial, spores did not need a wound to infect oak trunks. This mode of transmission, including spore production on foliar hosts, aerial transport in rain, survival in moist conditions such as rain-moistened trunks, and infection without wounds, may help explain the rapid spread of *P. ramorum* within a given geographical site.

Introduction

The newly discovered *Phytophthora ramorum* canker disease of oak (Sudden Oak Death Syndrome) threatens millions of acres of California woodlands where *Quercus agrifolia* (coast live oak), *Lithocarpus densiflorus* (tanoak), or *Quercus kelloggii* (black oak) are dominant species (McPherson *et al.* 2000, Garbelotto *et al.* 2001). 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.

On all known hosts to date, *P. ramorum* infects aerial parts of the plant. Infection on *Quercus* and *L. densiflorus* occurs on the main trunk and branches. Cankers have been noted as high as 20 m on the main trunk of *L. densiflorus*. On other hosts such as *Rhododendron*, *Arbutus menzesii* (madrone), *Manzanita*, and *Vaccinium ovatum* (huckleberry) *P. ramorum* infects stems and leaves. On *Umbellularia californica* (bay), *P. ramorum* is only found in the leaves. If infection is initiated on the aerial parts of plants, it follows that aerial movement of inoculum must occur. In addition, the observation of infected oak trees at all elevations on hillsides, not just valleys or stream courses, further suggests aerial spread of this pathogen. To explain this spatial pattern of disease, we need to understand the transmission biology of *P. ramorum*, consisting 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 spread of infection.

Identification of sources of spore production for *P. ramorum* in California oak woodlands is complicated by the presence of many host plant species in the forest. Currently, the host list includes 10 non-oak plant species in five plant families (Rizzo *et al.* unpublished

data). These other hosts may serve as sources of inoculum and act as infectious links among oak trees.

Production, movement, and survival of spores is likely to be highly affected by seasonal climatic changes in temperature and moisture in central coastal California. The current range of *P. ramorum* occupies areas with a Mediterranean climate influenced by maritime weather. Rainfall occurs during the cool winter months from December to April. During the summer, although morning fog is generally present, drought and high temperature conditions prevail in a significant portion of the range. Because production and survival of spores usually require very high humidity (Duniway 1983), abundance of *P. ramorum* spores may be highly seasonal and coincide with the winter rains. 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. However, *Phytophthora* spores also may survive adverse summer conditions as dormant resting spores residing in host tissue.

Once spores are produced and transported to oak trunks, suitable conditions are needed for survival of spores and infection of new host individuals. *Phytophthora* may infect through pruning wounds or naturally occurring wounds (Bostock and Doster 1985). However, in many cases, *Phytophthora* species do not need wounds to infect host plants.

In this paper, we provide data on a possible aerial pathway of infection for *P. ramorum* on oaks to explain the observed patterns of disease. We report on the types of spores produced by *P. ramorum* on tissue of several important hosts under laboratory conditions. We then monitor the abundance of spores in rainwater, soil, litter, and stream water in forest sites throughout the year. We test for the survival capabilities of spores under dry and moist conditions in the laboratory as a first attempt to understand survival potential on oak bark. We also test for survival of *P. ramorum* throughout the summer in living leaves of a wide-spread foliar host, *U. californica*. Finally, we perform a non-wounding inoculation on *L. densiflorus* and *Q. agrifolia* to investigate the potential of these spores to infect trees under natural conditions.

Methods

Production of spores

Production of spores was monitored on pieces of naturally infected host tissue collected from forest and nursery sites within the 300 km host range. Eight excised *Q. agrifolia* cankers, 9 infected *U. californica* 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 *Q. agrifolia* at weekly intervals from 22 April to 16 July, 2001, and plated on PARP medium to test for the presence of viable pathogens.

Movement of spores

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 *Q. agrifolia* and *U. californica* trees were infected with *P. ramorum* at this site. Raintraps consisted of a 165 cm x 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 2 February, 2001 and used to collect rainfall though winter, and for one unusually late rain on 27-28 June. 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. 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 µm 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 1/4 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 1/3 of the pear was immersed, and diH20 was added to the sample until 1/2 of the pear was immersed in water. Samples were allowed to remain 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 *Q. agrifolia* and *L. densiflorus* trees. Sampling ended in June when 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 half 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

Laboratory: 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 µl of suspension were added to either (1) 100 µl of diH20; (2) a filter moistened to saturation with 100 µl diH20; or (3) a dry filter. The moistened filters were kept in closed screw-cap tubes. An additional 150 µl 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 % 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.

Living U. californica leaves: To test for survival of *P. ramorum* inoculum in attached leaves of an important foliar host during the hot, dry summer, three infected leaves from each of 14 *U. californica* trees at the Marin Municipal Water District, Marin Co. were collected for isolation of *P. ramorum*. *P. ramorum* was isolated on pimaricin-ampicillin-rifampicin-PCNB agar (PARP). Collections began in July and occurred monthly. The number of positive leaves from each tree was recorded.

Non-wounding inoculation of Q. agrifolia *and* L. densiflorus

To test for the ability of spores to infect *Q. agrifolia* and *L. densiflorus* without an apparent wound in the bark, a non-wounding inoculation with spore suspensions of *P. ramorum* was carried out on the Marin Municipal Water District on 5 April 2000. For *Q. agrifolia*, 5 trees each were inoculated with spore suspensions from either isolate O-13 (from *L. densiflorus*) or O-16 (from *Q. agrifolia*), and 5 controls were inoculated with agar suspensions. For *L. densiflorus*, 4 trees each were inoculated with spore suspensions from either of the two isolates and there were 4 controls. The *L. densiflorus* trees were located in a coast redwood forest. The *Q. agrifolia* trees were located in a closed canopy, mixed-evergreen forest. Each of the inoculated trees received a sporangia suspension on one side and a chlamydospore suspension on the other side. Sporangia suspensions were made by placing 5 V8 agar mycelial plugs in 5 ml soil extract water at 18 – 23 ° C for 24 hours to induce sporangia production. (For methods, see Rizzo *et al.* 2002) The sporangia solutions averaged 180 spores / ml. Each chlamydospore solution was formed by blending a 6 cm diameter mycelial disk from isolates grown on CMA in a 100 x 15 mm petri dish for 6 weeks. Abundant chlamydospores were noted on the cultures before blending in 100 ml diH_2O. The chlamydospore solutions averaged 173 spores / ml. For the inoculation on each tree, the trunk was first wet with tap water. Treatments (O-13, O-16, control) were assigned at random to trees and 5 ml of sporangia suspension (or mock) and 100ml of chlamydospore suspension (or mock) were then applied to opposite sides of the tree in a 15 cm^2 patch. The inoculated area was then wrapped in plastic and sealed with duct tape at the top and bottom to maintain moisture. Gaps in the taped end were sealed with wet cotton. Plastic was removed after a week. Trees were checked on a

monthly basis for bleeding sap and canker formation.

Results

Production of spores

Spore production was observed on tissue of some host species. Sporangia were present on the surface of 3 of 9 infected *U. californica* 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

Raintraps: Rainwater contained viable spores of *P. ramorum* (Fig. 1). At some point during the sampling period, *P. ramorum* was recovered from rain captured at all 7 of the *Q. agrifolia* trees. Propagule counts were low, but they were present in rain water at each of the collection periods except for the brief summer storm in late June (13mm rainfall). Spore counts were variable from tree to tree and in positive samples ranged from 0.25-7 spores per liter. Of the six traps placed 5 m away from infected oak trees two were positive for *P. ramorum* during the sampling period.

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 positve, 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

Laboratory: 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% rh at room temperature in the 'dry filter' treatment (data not shown). Chlamydospores survived better than zoospores in both the water and moist filter treatments. Zoospore survival averaged less than 20% at the end of the 30 day period while chlamydospore survival at 30 days in water and on moist filters still averaged 75% and 41% of starting values, respectively.

Living U. californica *leaves:* Inoculum of *P. ramorum* survived 4 months of hot, drying conditions in *U. californica* leaves. Inoculum levels did not decrease with time. The number of positive leaves for *P. ramorum* were 15, 17, and 20 out of 42 for July, August, and September, respectively.

Non-wounding inoculation of Q. agrifolia *and* L. densiflorus

Five weeks post inoculation, one *Q. agrifolia* and one *L. densiflorus* exhibited bleeding symptoms. Five months after the inoculation, two *Q. agrifolia* and two *L. densiflorus* showed bleeding symptoms. Other *L. densiflorus* may also be positive, yet not initially show bleeding, as determined by an earlier wound inoculation experiment (Rizzo *et al.* 2002). On all four infected trees, bleeding symptoms appeared on the side of the tree inoculated with sporangia. Three of the positive inoculations were caused by O-16 (from *Q. agrifolia*) and one by O-13 (from *L. densiflorus*

Discussion

Rapid production of spores on foliar hosts, such as *U. californica* and *Rhododendron*, may be the 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 *U. californica* and *Rhododendron*, the two foliar hosts tested. Chlamydospores were also noted on infected *U. californica* leaves. In addition, we have observed chlamydospores on infected *Rhododendron* leaves in moist chambers for other experiments.

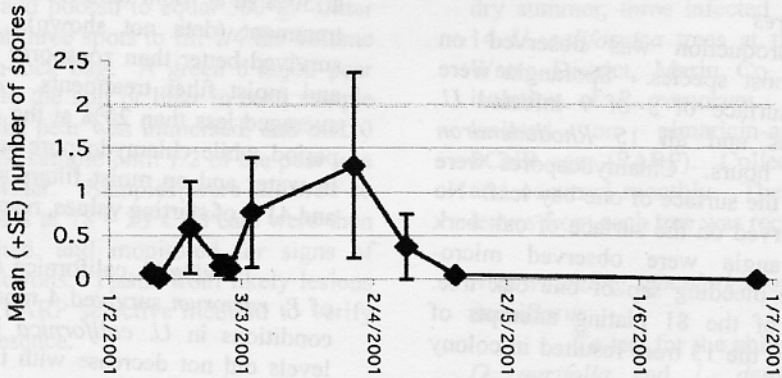

Fig. 1. Mean number of *P. ramorum* spores per liter of rainwater (± standard error) for storms occurring from February through June, 2001 at the Fairfield Osborn Preserve, Sonoma Co. Means were based on the average from two collecting traps at each of seven *Q. agrifolia* trees.

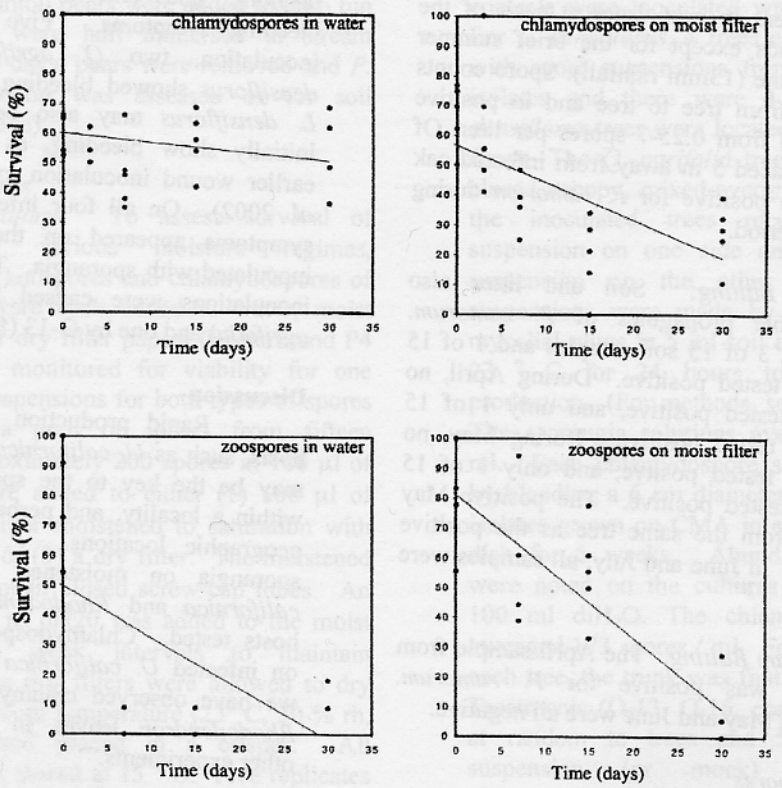

Fig. 2. Survival over time of *P. ramorum* chlamydospores and zoospores in water and on moist filter paper at 15 C. Each datum represents the spore count of one of five treatment replicates plated to agar at a given time point. Spore count data is scaled as a percent of the highest spore count at time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

While rhododendron species are not major components of most California forests, *U. californica* is a dominant species in many coast live oak forest types. Consistent with laboratory results on spore production, evidence from the field suggests that inoculum from *U. californica* leaves may be very important in vectoring *P. ramorum* to oaks. Swiecki (2001) found a significant association between infected oaks and the presence of *U. californica* trees. Rainwater traps in this study placed 5 m from infected *Q. agrifolia* contained viable spores that may have come from overstory infected *U. californica* trees. Furthermore, we often observe an increase in *Phytophthora*-like leaf spots on *U. californica* growing within oak infection centers. To further investigate the importance of *U. californica* as an inoculum source, studies are underway to determine temperature requirements for sporangia production on *U. californica* leaves, the viability of *P. ramorum* in leaves attached to trees or fallen in litter, and the distance spores can travel from *U. californica* 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, unpublished data), 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 abundance of viable propagules in these media 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 likely necessary for production of spores that eventually fall down to 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% 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. Thus spores transported to the trunks of oak trees may survive for significant periods of time during the wet winter months. 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.

Survival of *P. ramorum* in leaves of *U. californica* trees throughout the hot, dry summer may allow relatively high levels of inoculum to persist in forests even though soil, litter, or ephemeral streams may no longer harbor viable spores. The presence of inoculum may allow for rapid spread of *P. ramorum* from many leaf surfaces once winter rains begin, and lead to an exponential spread of infection among the numerous host plants.

P. ramorum does not need wounds to infect *Q. agrifolia* and *L. densiflorus* trees. If trunks stay wet during winter months with rains occurring every few days, spore survival times of up to 30 days in moist micro-habitats such as grooves in bark or beds of moss would allow for a long window of opportunity to initiate infection. Although some *L. densiflorus* in this experiment may be infected yet fail to show bleeding symptoms, it appears that not all trees became infected in this trial even though inoculum levels were quite high. This raises the hope that some type of resistance is present at the onset of infection.

Given the ability of *P. ramorum* to produce spores on foliar hosts which can then be

carried in wind-blown rain, survive on oak trunks in moist conditions, and infect oak trees without a wound, 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 such as the 300 km gap between Mendocino, California and Brookings, Oregon. 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). Although anthropomorphic spread of infection cannot be ruled out, it 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

Bostock RM, Doster MA (1985) Association of *Phytophthora syringae* with pruning wound cankers of almond trees. *Plant Disease* **69**, 568-571.

Brasier CM, Kirk SA (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 JM (1983) Role of physical factors in the development of *Phytophthora* diseases. In '*Phytophthora*: Its biology, taxonomy, ecology and pathology'. (Eds CC. Erwin, S Bartnicki-Garci and P H Tsao) pp. 175-188. (American Phytopathological Society, St. Paul, Minnesota)

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

Hansen E M, Goneen DJ, Jules ES, Ullian (2000) Managing Port-Orford Cedar and the introduced pathogen *Phytophthora lateralis*. *Plant Disease* **84,** 4 – 12.

McPherson BA, Wood DL, Storer AJ, Svirha P, Rizzo D M, Kelly NM, Standiford RB (2000) 'Oak mortality syndrome: Sudden death of oaks and *L. densiflorus*'. (California Department of Forestry. Tree Note No. 26)

Ristaino JB, Gumpertz. MC (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 DM, Garbelotto M, Davidson JM, Slaughter GW, Koike S (2002) A new *Phytophthora* canker disease associated with extensive mortality of *Lithocarpus* and *Quercus* in California. *Plant Disease* **86,** 205-214.

Swiecki T Bernhardt E (2001) Evaluation of stem water potential and other tree and stand variables as risk factors in *Phytophthora* canker development in *Q. agrifolia* and *L. densiflorus*. Report to the USDA Forest Service.