

# Pathogenicity Variation in Two West Coast Forest *Phytophthoras*, *Phytophthora nemorosa* and *P. pseudosyringae*, to Bay Laurel<sup>1</sup>

R.E. Linzer<sup>2</sup> and M. Garbelotto<sup>2</sup>

## Abstract

Two recently described pathogenic oomycetes, *Phytophthora nemorosa* and *P. pseudosyringae*, have overlapping host and geographic ranges in California and Oregon forests with *P. ramorum*, causal agent of “sudden oak death” disease. Preliminary genetic evidence indicates *P. nemorosa* and *P. pseudosyringae* may be introduced in this region; their sympatric distribution with *P. ramorum* suggests they may be an interacting factor in *P. ramorum* disease.

The ultimate goal of this project is to characterize the outcome of interaction during infection by *P. ramorum*, *P. nemorosa* and *P. pseudosyringae* in the epidemiologically important host, bay laurel (*Umbellularia californica*). Experiments will account for variability in host, pathogen and environment, and leaf lesion area in detached bay leaves will be used as the proxy for pathogenicity. Therefore, the proximal goals of this portion of the project are to describe pathogenicity variability and refine methods for leaf infection. Here, we describe four preliminary experiments conducted to select *Phytophthora* isolates for inoculation and to optimize leaf infection methods.

1) In order to identify a subset of *Phytophthora* isolates with sufficient sporangia production for infection and to determine the liquid medium yielding highest sporangial concentration, we screened 15 California and Oregon isolates of each *Phytophthora* species for sporangia formation. All isolates were originally recovered from *U. californica*. Agar disks, 5 mm in diameter, were excised from the margin of *Phytophthora* colonies growing on V8 agar and placed in the bottom of separate wells in 24-well micro-titer plates. Three replicates of each isolate were incubated in each of three liquid media: distilled water and two-percent infusions of soil or *U. californica* leaves in distilled water. Liquid media was added until even with the top surface of each disk, and plates were incubated for five days. Maximal production of sporangia was achieved in the soil-water infusion for all *P. nemorosa* isolates. Though sporulation varied by medium for *P. ramorum* and *P. pseudosyringae*, consistent high sporangial production was also achieved in two-percent soil-water infusion. Thus, we will proceed with the soil-water infusion as the induction medium for subsequent experiments. Isolate sets of each species were reduced to the 10 highest sporulators, excluding isolates with sparse or absent sporangia.

2) Next, we tested for zoospore release from the selected 10 sporangia-producing isolates of each species. Agar plugs from colonies growing on V8 medium were incubated with soil-water

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<sup>1</sup> A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

<sup>2</sup> University of California, Berkeley, Dept. of Environmental Science, Policy, and Management, 137 Mulford mailcode# 3114, Berkeley, CA 94720.  
Corresponding author R. Linzer, [rwarner@nature.berkeley.edu](mailto:rwarner@nature.berkeley.edu).

added up to the plug surface. *P. ramorum* isolates were incubated for three days at 18°C and *P. nemorosa* and *P. pseudosyringae* for five days at 14 and 18°C, respectively. To induce zoospore release, agar plugs and induction medium were incubated at 4°C for 30 minutes then for 45 minutes at room-temperature before zoospore quantification. Though all isolates yielded abundant sporangia, zoospore release was extremely variable. Release was plentiful from all *P. ramorum* and some *P. pseudosyringae* isolates but absent or too low to count in all *P. nemorosa* and the remaining *P. pseudosyringae* isolates.

3) To allow for zoospore release from all isolates, we then tested different induction procedures, comparing the standard *P. ramorum* release parameters, above, to two cycles of temperature shock and a temperature shock cycle of 30 minutes at 4°C followed by a room-temperature recovery time of 2.5 hours. Although the two temperature shocks yielded zoospore numbers indistinguishable from the standard protocol, increasing the recovery time resulted in a sufficient zoospore concentration to undertake subsequent inoculations.

4) To further refine leaf inoculation methods, we used a subset of two isolates of each species and compared two detached-leaf inoculation methods: a zoospore-suspension drop on the abaxial leaf surface vs. dipping leaf tip in a zoospore suspension. As *P. nemorosa* has a lower optimal *in vitro* growth temperature than do *P. ramorum* and *P. pseudosyringae*, we added the effect of environmental variability on infection outcome by undertaking the experiment in parallel at two temperatures: 12 and 18°C. For the abaxial leaf drop method, detached leaves of approximately the same age from a single bay host were infected by dropping 50 µl of a  $2 \times 10^4$  zoospores/mL suspension on the abaxial leaf surface one cm from the tip of the leaf. Alternately, for the leaf dip method, leaves were infected by tip-down immersion in a 50 mL conical tube containing 300 µl of a  $2 \times 10^4$  zoospores/mL suspension and removed from the suspension after 24 hours. Leaves were incubated on 1 cm-gridded racks in partially sealed plastic boxes lined with moist paper towels. Leaves were misted every two days and harvested on the ninth day. Resulting leaf lesion area was quantified using APS Assess. Both inoculation methods resulted in measurable lesions, and lesions developed at both temperatures for five of the six isolates. The trend was toward larger lesions at 18°C for all species, though most differences were not significant. Many drop lesions were significantly larger than lesions resulting from leaf dips. Since the leaf area exposed to inoculum can be more easily standardized using the leaf drop method, we will use this method in subsequent experiments.

Using methods and isolates determined above, we will compare pathogenicity of the 10 isolates of each of the three species identified in step 2) at three temperatures: 12, 18 and 24°C, against leaves of one intermediately susceptible bay laurel host. The range of pathogenicity variability thus identified will be utilized to select isolates for competition experiments.

Key words: *Umbellularia californica*, detached leaf, zoospore, sporangia, inoculation.