Phytophthora ramorum: an emerging pathogen

M. Garbelotto[1], D.M. Rizzo[2], J.M. Davidson[2], K. Ivors[1], P.E. Maloney[2], D. Hüberli[1], K. Hayden[1], T. Harnik[1] and S.T. Koike[3]
[1] University of California, Berkeley, [2] University of California, Davis, [3]University of California Cooperative Extension *Email: Matteo@nature.berkeley.edu*

Distribution in Oregon and California

Aided by taxon-specific PCR primers, we have determined that *Phytophthora ramorum*, causal agent of Sudden Oak Death, is not restricted to oaks, but has a host range encompassing at least 11 families and 18 plant species, including trees, understory shrubs, and herbaceous plants. While forest *Phytophthora* species are commonly known to cause root diseases, *P. ramorum* appears to exclusively cause above ground disease symptoms and can be dispersed aerially. Analysis of North American isolates using amplified fragment length polymorphisms (AFLPs) indicates largely a clonal population, and that the European and North American populations are quite distinct. Our results highlight the power of DNA-based diagnostics.

Zoospore release

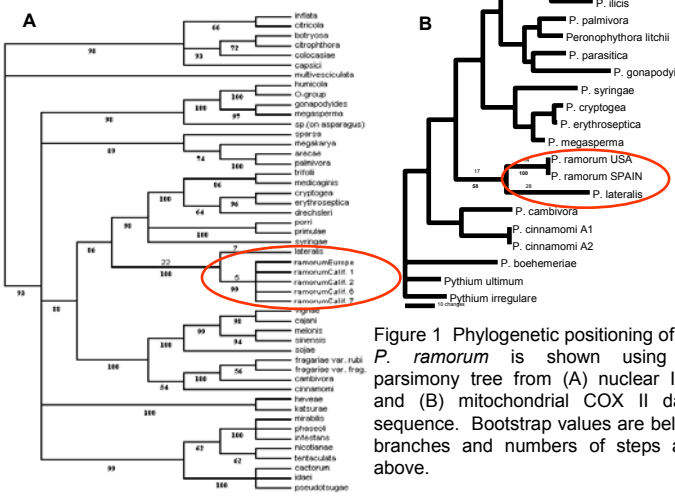


Figure 1 Phylogenetic positioning of *P. ramorum* is shown using a parsimony tree from (A) nuclear ITS and (B) mitochondrial COX II data sequence. Bootstrap values are below branches and numbers of steps are above.

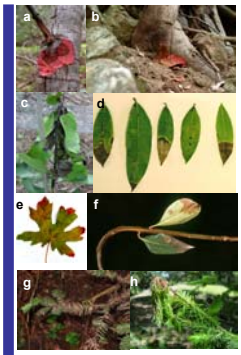


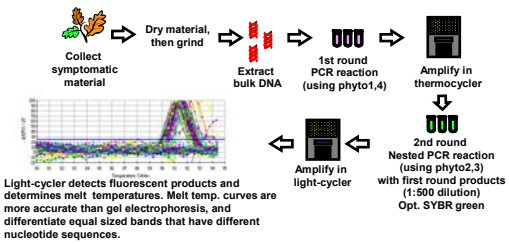
Figure 1. Symptoms of infection by *P. ramorum* on various hosts (A) phloem canker on main trunk of *Lithocarpus densiflora*, (B) stem canker on *L. densiflora* stops at the soil line, (C) *Arbutus menziesii* with foliar and stem lesions, (D) *Umbellularia californica* with leaf tip necrosis, (E) *Acer macrophyllum* with marginal leaf scorch, (F) branch dieback and foliar lesions on *Rhododendron* sp., (G) *Sequoia sempervirens* with needle necrosis on understory sapling, (H) *Pseudotsuga menziesii* with tip wilting due to branch cankers.

Host	Common name	Plant part infected	Original detection method
Quercus agrifolia (Fagaceae)	Coast live oak	main stem	Culture
Q. kellogii (Fagaceae)	California Black oak	main stem	Culture
Q. parvula var. *shrevei* (Fagaceae)	Shreve's oak	main stem	Culture
Lithocarpus densiflora (Fagaceae)	Tanoak	main stem, branches, leaves	Culture
Arbutus menziesii (Ericaceae)	Madrone	branches, leaves	PCR
Vaccinium ovatum (Ericaceae)	Evergreen huckleberry	main stem, branches, leaves	Culture
Arctostaphylos manzanita (Ericaceae)	Manzanita	branches, leaves	PCR
Rhododendron sp. (Ericaceae)	Ornamental rhododendron	branches, leaves	Culture
Rhododendron macrophyllum (Ericaceae)	Rhododendron	branches, leaves	PCR
Umbellularia californica (Lauraceae)	Bay laurel, Oregon myrtle	leaves	PCR
Acer macrophyllum (Aceraceae)	Big leaf maple	leaves	PCR
Heteromeles arbutifolia (Rosaeceae)	Toyon	branches, leaves	PCR
Aesculus californica (Hippocastanaceae)	California buckeye	branches, leaves	PCR
Rhamnus californica (Rhamnaceae)	Coffeeberry	branches, leaves	PCR
Lonicera hispidula (Caprifoliaceae)	Honeysuckle	leaves	PCR
Sequoia sempervirens (Taxodiaceae)	Coast redwood	branches, leaves	PCR
Pseudotsuga menziesii (Pinaceae)	Douglas-fir	branches, leaves	Culture

Based on this data, the closest relative to *P. ramorum* is *P. lateralis*. The two species share similar temperature optima (approx. 18^{0}C), and some morphological traits. However, *P ramorum* has a broad host range and is aerial, whereas *P. lateralis* has only one host and is a soil borne pathogen.
The sequence data used for the trees above was used to design specific PCR primers (see below).

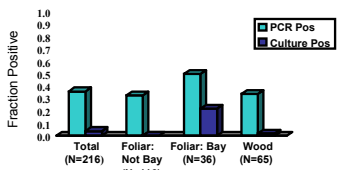
- *P. ramorum* causes two different types of diseases: non-lethal foliar and twig infections (e.g. on bay laurel, buckey, maple) and lethal branch or stem infections (e.g. on oaks, tanoak).
- Foliar infections play a key role in the epidemiology of *P. ramorum* by serving as a source of inoculum which is then spread aerially through rainsplash. Sporangia are rarely observed on infected stems of oaks and tanoaks.

Overview of PCR method



Light-cycler detects fluorescent products and determines melt temperatures. Melt temp. curves are more accurate than gel electrophoresis, and differentiate equal sized bands that have different nucleotide sequences.

Culture versus nested PCR



Nested PCR was more sensitive than culturing (P <0.001). For both culturing and PCR, success rate varies by type of sample (P=0.0036). Bay leaf samples processed were those from which it was difficult to culture *Phytophthora*, thus biasing comparison in favor of PCR.

A PCR assay was designed using primers specific for *P. ramorum*. No cross reactivity with other species was detected, except when using extremely high concentration of pure *Phytophthora* DNA.

Of the 13 non-oak host species we have identified in California, 10 were first detected using PCR amplification from infected plant material (Table 1).

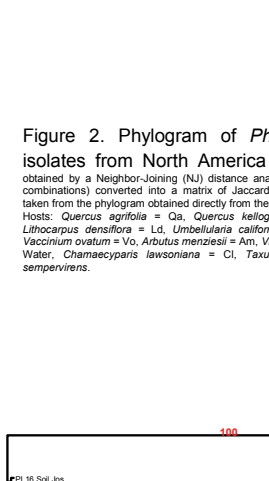


Figure 2. Phylogram of *Phytophthora ramorum* isolates from North America and Europe. Tree was obtained by a Neighbor-Joining (NJ) distance analysis of AFLP binary data (4 primer combinations) converted into a matrix of Jaccard's similarity. Bootstrap values were taken from the phylogram obtained directly from the NJ analysis of binary data.
Hosts: *Quercus agrifolia* = Qa, *Quercus kelloggii* = Qk, *Quercus parvula* = Qp, *Lithocarpus densiflora* = Ld, *Umbellularia californica*= Uc, *Rhododendron* spp.= Rh, *Vaccinium ovatum* = Vo, *Arbutus menziesii* = Am, *Viburnum* spp. = Vb, Recycling water = Water, *Chamaecyparis lawsoniana* = Cl, *Taxus brevifolia* = Tb, Ss = *Sequoia sempervirens*.

Based on AFLP data:
- European and North American populations of *P. ramorum* are distinct. Although both populations display limited genetic variability, to date all European isolates represent different genotypes, while a single genotype is representative of over 80% of all North American isolates.
- The dominant North American genotype has been found on most known hosts suggesting there is no host preference by isolates.