

The Search for the Origin of *Phytophthora ramorum*: A First Look in Yunnan Province, People's Republic of China¹

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Abstract

Phytophthora ramorum, a previously unknown pathogen, was first seen in Europe on ornamental nursery stock in the early 1990s (Werres and others 2001). It has since been implicated in the death of thousands of oaks (*Quercus agrifolia* and *Q. kelloggii*) and tanoaks (*Lithocarpus densiflorus*) in the western United States (Rizzo and others 2002). Although populations of *P. ramorum* in Europe and the United States are largely distinct from one another, the organism is considered invasive in both locations with an operating hypothesis that it was introduced on both continents (Brasier and others 2004). It is possible that *P. ramorum* may have originated somewhere in Asia and was unknowingly transported on commercial or privately collected ornamental plants. Asia is a center of diversity for many genera of plants that are *P. ramorum* hosts. More specifically, northern Yunnan Province in the People's Republic of China has an abundance of oaks and tanoaks in a variety of forest ecosystems. Rhododendrons and azaleas, among others, have been widely collected in this region for at least 150 years by plant enthusiasts from across the globe. Furthermore, the climatic conditions in this part of the Province correlate well with those predicted to be favorable for *P. ramorum*.

The International Forestry, Forest Health Protection, and Vegetation Management and Protection Research staffs of the Forest Service, U.S. Department of Agriculture, have been working cooperatively with forestry officials in China over the past several years on a variety of issues related to forest insects and pathogens. In August 2004 a small team of USDA Forest Service scientists traveled to Yunnan Province in southwestern China to begin collaboration with scientists from the Chinese Academy of Forestry (CAF) on projects investigating forest *Phytophthora* species in China with the more immediate goal of initiating a survey specifically for the pathogen *P. ramorum*. International Forestry, using its network of contacts in China, arranged for field personnel in China to assist with site identification, visits and sampling.

We visited four forest sites where a variety of species of *P. ramorum* host genera occur. Three sites were in Diqing Prefecture in the vicinity of the city of Zhongdian (Shangri-la) in northwestern Yunnan Province. Plant associations on these sites included evergreen oak (*Quercus*), *Larix*, and *Picea* stands

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with *Rhododendron* understories, evergreen oak and pine (*Pinus*) stands, and pine forests with *Lithocarpus* species and other oak species in the understory. Sites ranged in elevation from approximately 2000 m to 3500 m. Genera of other *P. ramorum* hosts were also present including *Lonicera* and *Rosa*. We also visited a site outside of the city of Kunming in central Yunnan province. Forests there were composed of several *Quercus* species with *Lithocarpus* species and *Rhododendron* species in the understory.

We observed foliar and dieback symptoms similar to those associated with *P. ramorum* in the United States and Europe on a variety of plants. Symptoms included inner bark necrosis, shepherd's crooking of small diameter stems and new growth, leaf tip dieback, leaf margin necrosis, and leaf spotting. Symptomatic tissues were collected in the field, plated on CARP media in the evenings after collection, and then transported to and processed in the CAF pathology laboratory in Beijing for sub-culturing and DNA extraction. In Beijing, mycelium and plant tissues contained within a single plate were pooled together and DNA was extracted using a standard CTAB-based extraction protocol. We also extracted DNA from an additional 30 symptomatic plant tissue samples. The DNA assay performed while in Beijing was inconclusive and DNA samples were brought back to the United States. All DNA samples were further purified and amplified using the ITS 6 and 7 rDNA primers developed specifically for use on Oomycetes. Out of 92 total samples, 29 samples amplified at least a single visible band based on ethidium bromide-stained gels. Due to the manner in which samples were taken and the redundancy of band profiles across some samples, only 19 bands from 18 samples were subject to further analysis. *P. ramorum* was not immediately identified from these samples. We did find DNA sequence BLAST search matches with *Cryptococcus flavescens* and *Mortierella alpina* for some samples. The possibility that other *Phytophthora* species are involved in symptom development is currently under investigation.

The forests we visited in Yunnan Province were remarkably similar in genera, yet profoundly different in terms of species diversity and composition relative to forests on the west coast of the United States where *P. ramorum* is currently found. Symptoms similar to those caused by *P. ramorum* on wildland and nursery stock target species (*Quercus*, *Lithocarpus*, *Rhododendron*) in the western United States, as well as minor *P. ramorum* hosts (such as *Lonicera*, *Smilacina*), were abundant. While our laboratory results were not definitive, the team sees promise for additional investigations and received an enthusiastic response from our Chinese colleagues for continued collaboration. Further field work in Yunnan Province may include sampling additional hosts and forest types, as well as sampling forest soil and water. Understanding the origin of a presumably introduced pathogen, such as *P. ramorum*, will help us better understand its biology, ecology, pathology, and genetics, and may lead to management actions that minimize its impact.

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