Some Challenges of Recognizing Invasive *Phytophthoras* and Finding Their Origins[1]

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Introduction

Discovering the origins of *Phytophthora ramorum* remains a challenge. To improve our chances of finding the origin of *P. ramorum* or any other introduced organism, we need to be sure of our motivation, because success will require persistence. We need to be able to distinguish indigenous from exotic organisms, to know what to look for and recognize when we have found it, and we need a practical "search image" to guide the discovery and sorting of candidate organisms.

Perhaps the most frequently asked question about *P. ramorum* and sudden oak death is "Where did it come from?" Despite several expeditions to far off lands (Goheen and others 2005) and repeated searches closer to home, the answer is unchanged: "I don't know. Maybe China." Our ignorance is frustrating, but shouldn't be surprising. We actually know the answer for very few pathogens. Perhaps it is timely to reexamine our approach. To improve our chances of finding the origin of *P. ramorum* or any other introduced organism, we need to deal with three additional questions: "How do we know it was introduced in the first place?" "Why do we need to know?" and "Where should we look and what should we be looking for?" The first is a necessary challenge to our epidemiological and genetic assumptions about invasive species of pathogenic fungi. The second is a question of motivation and commitment, and "Where to look?" is as much a question of opportunity as biology.

Exotic or Indiginous?

The question is not as trivial as it may sound. Sometimes the actual introduction of an organism or the beginning of an epidemic is noted, and there is sufficient documentation to be sure that it truly is a new phenomenon. More often, we can't be sure that the apparent novelty isn't an artifact of no one looking before.

In the absence of clear "trace-back" information on origins, we usually invoke two lines of evidence to separate native from introduced pathogens. We suppose that native pathogens have evolved with their host plants in a particular ecosystem, with checks and balances to prevent catastrophic epidemics. Certainly we don't expect that an indigenous pathogen would threaten the evolutionary reproductive potential of a population of a host species. We also suppose that populations of native pathogens will exhibit more genetic diversity than introduced organisms that likely exploded from one or a few individuals that were first established. In the case of

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heterothallic organisms, with two mating types, we expect to find both in the region of origin.

Phytophthora ramorum provides a relatively straightforward test of these assumptions. It appeared recently and suddenly, in a populated area with an abundance of plant pathologists, affecting an iconic and abundant tree (coast live oak, *Quercus agrifolia*) (Rizzo and others 2005). Sudden oak death has a clear beginning in both California and in Oregon, and it continues to spread (Hansen and others 2008) (table 1). Genetic markers allow us to track its further spread around the world and within North America (Goss and others 2009, Hansen and others 2003, Ivors and others 2004, Mascheretti and others 2008, Prospero and others 2009, Werres and others 2001). *P. ramorum* kills trees of all ages in expanding areas of mortality. Tanoaks (*Lithocarpus densiflorus*) are especially vulnerable, and their regional survival is threatened by this pathogen. *P. ramorum* did not coevolve with tanoak or coast live oak. Introduced organisms do not necessarily cause dramatic epidemics, however.

Table 1—Retrospective detection of dead tanoaks on color aerial photographs at five sites in Oregon where *P. ramorum* was confirmed as cause of death in 2001 (Hansen and others, 2008)

	Number of symptomatic tanoak trees visible on color aerial photos			
Site	**1996**	**1998**	**2000**	**2001**
10	0	1	7	110
11	0	0	4	31
17	0	0	1	12
18	0	0	1	14
33	0	0	1	32

Phytophthora ramorum is heterothallic (Werres and others 2001), but only one mating type (A2) is present in North American forests and only the opposite mating type (A1) is present in Europe (Ivors and others 2004). Neither population is sexually reproducing; the origin must be elsewhere. Presence of only one mating type is one kind of evidence for a genetic “bottleneck.” In many cases, introduction must have involved only one or a few individuals, and the resulting epidemic population will have a correspondingly narrow genetic diversity, especially if it is limited to clonal reproduction, as is *P. ramorum* when only one mating type is present.

Measuring genetic diversity requires many isolates of a population, and demonstrating a “bottleneck” requires information on the diversity of the originating population as a basis for comparison. Although “limited genetic diversity” is often cited in support of a conclusion that a *Phytophthora* species is of exotic origin (Cooke and others 2005, Linzer and others 2009, Ivors and others 2004), this evidence is actually very difficult to evaluate. DNA fingerprinting using “AFLP” markers is often used, but differences in sample sizes, sources, and statistical treatments prevent comparisons between studies. A study of *P. quercina* in Europe for example, found all isolates to be closely related, but each was genetically unique. A similar study of *P. nemorosa* in California found that most isolates had identical DNA fingerprints. Both studies concluded that the organisms were introduced, but in neither case was an indigenous population used for comparison. The interpretation is still more difficult because these examples are both homothallic organisms, and inbreeding would be expected to narrow genetic diversity, even in the area of origin.

Why Look?

"Why do we need to know where it came from?" is a question of motivation. Can we justify the expense and the mental and physical exertion necessary to undertake the search? It is fun to go fishing for *Phytophthora* in exotic places. It usually doesn't take much persuasion to get plant pathologists to sign up for such expeditions, but entertainment value doesn't appear on many grant proposals. Biosecurity is the most common official justification. But will we act on the information, strengthening quarantine regulations for example, or will global politics and economics trump risks to domestic agriculture and forestry yet again? There are other important uses for this information, however. Discovering origins is fundamental to basic studies of evolution and speciation. It is necessary for testing underlying ecological assumptions and theories of population genetics and coevolution. Again, *P. ramorum* illustrates these points.

The threat posed by *P. ramorum* to forests and woody plant horticulture around the world is clear. It is a known, named threat to global biosecurity, and quarantine regulations are in place to halt its movement to new areas. Knowledge of its lands of origin, however, will add only marginally to the effectiveness of these regulations. On the other hand, recent work on the population genetics of this dangerous pathogen (Goss and others, unpublished) suggests a complex evolutionary history, and studies of indigenous populations, once found, will be very revealing of the processes of isolation and speciation that lead to such complex pathogenic potential.

Phytophthora ramorum, as we know it today in North America and Europe, is comprised of three reproductively isolated clonal lineages, NA 1, NA 2, and EU 1 (Figure 1). Goss and colleagues concluded that:

- *P. ramorum* lineages are descended from a sexually reproducing population.
- The age of the three lineages is estimated to be between 1.5 and 5.4 million years.
- The lineages have probably been separated for about one tenth of their evolutionary history.

Thus the most recent split between lineages (NA2 from NA1) was a minimum of 165,000 years ago.

The ancestral, sexually reproducing population may not even exist anymore! We are probably looking for three separate origins for the three lineages.

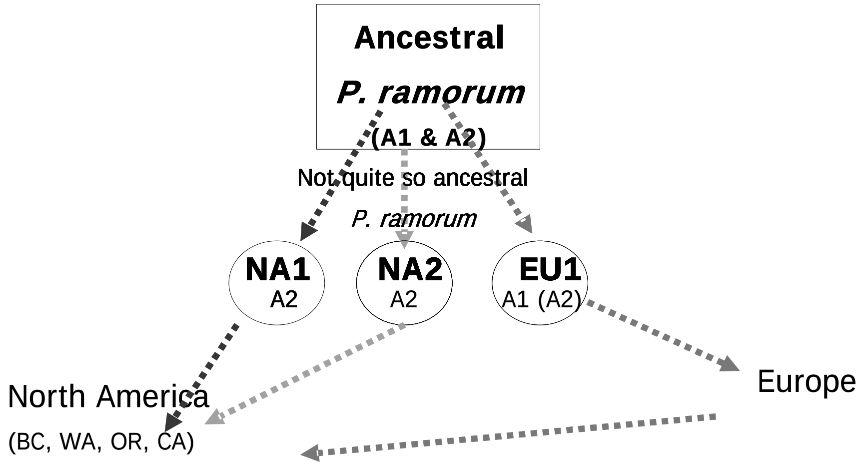

Figure 1—Hypothetical origins of *Phytophthora ramorum* as we understand it today.

Where to Look?

We usually jump right into the question "Where should we look for the source population of (for example) *P. ramorum*?" A typical analysis starts with a host - *P. ramorum* is virulent on rhododendron - and jumps into an expedition. So let's go look where rhododendrons come from (Goheen and others 2005). A better first step, however, may be to check existing culture collections and DNA sequence databases for unidentified or misidentified isolates. The conspecificity of *P. ramorum* in Europe and North America was first suspected based on morphological similarity between the new fungus from California, and an unidentified isolate in Germany. Similarly, *Phytophthora* isolates closely resembling *P. kernoviae* were found among old unidentified isolates in New Zealand, and new, lethal outbreaks of *Phytophthora* on alder in Australia and Foster City California were matched to the new species *P. siskiyouensis* through GenBank (Rooney-Latham and others 2008).

The so-called "alder *Phytophthora*" provides another example. *P. alni* was isolated from dying alders in Europe and shown to be cause of a dramatic new disease. It appeared to be a new, exotic, invasive pathogen based largely on its apparent sudden appearance and the destruction it caused. A search of the genetic databases showed it to be unique, but closely related to well-known organisms. Further genetic investigation demonstrated that *P. alni* is a hybrid species of complex parentage (Brasier and others 2004, Ioos and others 2006). Very recent surveys of remote streams and soils associated with wild alder stands in Alaska have yielded one of the putative parents (Adams and Trummer, this proceedings). The new isolates do not appear to be causing any dramatic disease in Alaska; this indeed may be at least one of the "origins" of the alder *Phytophthora*.

Literature Cited

Brasier, C.M.; Kirk, S.; Delcan, J.; Cooke, D.E.I.; Jung, T. and Man in't Veld, W.A. 2004. *Phytophthora alni* sp. nov. and its variants: designation of emerging heteroploid hybrid pathogens spreading on *Alnus* trees. Mycological Research. 108: 1172–1184.

Cooke, D.E.L.; Jung, T.; Williams, N.A.; Schubert, R.; Osswald, W. and Duncan, J.M. 2005. Genetic diversity of European populations of the oak fine-root pathogen *Phytophthora quercina*. Forest Pathology. 35: 57–70.

Goheen, E.; Kubisiak, T. and Zhao, W. 2005. The Search for the origin of *Phytophthora ramorum*: a first look in Yunnan Province, People's Republic of China. In: Frankel, S.J.; Shea, P.J.; Haverty, M.I., tech. coord. Gen. Tech. Rep. PSW-GTR-196. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station: 113–115.

Goss, E.M.; Carbone, I. and Grünwald, N.J. 2009. Ancient isolation and independent evolution of the three clonal lineages of the exotic sudden oak death pathogen *Phytophthora ramorum*. Molecular Ecology. 18: 1161–1174.

Goss, E.M.; Larsen, M.; Chastagner, G.A.; Givens, D.R. and Grünwald, N.J. 2009. Population genetic analysis infers migration pathways of *Phytophthora ramorum* in US nurseries. PLoS pathogens 5(9): e1000583. doi:10.1371/journal.ppat.1000583.

Hansen, E.M.; Kanaskie, A.; Prospero, S.; McWilliams, M.; Goheen, E.M.; Osterbauer, N.; Reeser, P. and Sutton, W. 2008. Epidemiology of *Phytophthora ramorum* in Oregon tanoak forests. Canadian Journal of Forest Research. 38: 1133–1143.

Hansen, E.M.; Reeser, P.W.; Sutton, W. and Winton, L.M. 2003. First report of A1 mating type of *Phytophthora ramorum i*n North America. Plant Disease. 87(10): 1267.

Ioos, R.; Andrieux, A.; Marcais, B. and Frey, P. 2006. Genetic characterization of the natural hybrid species *Phytophthora alni* as inferred from nuclear and mitochondrial DNA analyses. Fungal Genetics and Biology. 43: 511–529.

Ivors, K.L.; Hayden, K.; Bonants, P.J.M.; Rizzo, D.M. and Garbelotto, M. 2004. AFLP and phylogenetic analyses of North American and European populations of *Phytophthora ramorum*. Mycological Research. 108(4): 378–392.

Linzer, R.; Rizzo, D.; Cacciola, S. and Garbelotto, M. 2009. AFLPs detect low genetic diversity for *Phytophthora nemorosa* and *P. pseudosyringae* in the US and Europe. Mycological Research. 113: 298–307.

Mascheretti, S.; Croucher, P.J.P.; Vettraino, A.; Prospero, S. and Garbelotto, M. 2008. Reconstruction of the Sudden Oak Death epidemic in California through microsatellite analysis of the pathogen *Phytophthora ramorum*. Molecular Ecology. 17: 2755–2768.

Prospero, S.; Grünwald, N.J.; Winton, L.M. and Hansen, E.M. 2009. Migration patterns of the emerging plant pathogen *Phytophthora ramorum* on the west coast of the United States of America. Phytopathology. 99: 739–749.

Rizzo, D.M.; Garbelotto, M. and Hansen, E. 2005. *Phytophthora ramorum*: integrative research and management of an emerging pathogen in California and Oregon forests. Annual Review of Phytopathology. 43: 309–335.

Rooney-Latham, S.; Blomquist, C.L.; Pastalka, T. and Costello, L. 2009. Collar rot on Italian alder trees in California caused by *Phytophthora siskiyouensis*. Online. Plant Health Progress. DOI: 10.1094/PHP-2009-0413-01-RS.

Werres, S.; Marwitz, R.; Man in 't Veld, W.A.;. De Cock, A.W.; Bonants, P.J.M.; De Weerdt, M.; Themann, K.; Ilieva, E. and Baayen, R.P. 2001. *Phytophthora ramorum* sp. nov: a new pathogen on Rhododendron and Viburnum. Mycological Research. 105(10): 1155–1165.