

TITLE: Early detection of *P. ramorum* lineages in Oregon forests using genetic markers.

LOCATION: Western Oregon

DATE: September 2013

DURATION: Year 2 of 3-year project.

FUNDING SOURCE: Fire

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PROJECT OBJECTIVES: To enhance the early detection of *P. ramorum* in forests by using genetic markers (microsatellites or SSRs) to identify clonal lineages. Specific objectives include:

- A. Determine genotypes of important current infestations, particularly “outliers” such as Wheeler creek and Cape Sebastian, and isolated infestations found by stream baiting, and compare them to existing forest and nursery genotypes (Year 1)
- B. Update the SSR forest database to allow comparison with Nik Grunwald’s worldwide nursery data base and the California databases (Year 1).
- C. Maintain the capacity for continued genotyping of isolates from any new infestations that occur over the next few years (Years 2 & 3 - this would be at a reduced funding level compared to the first year).

JUSTIFICATION:

a. Linkage to FHM program. Early detection of *P. ramorum* in Oregon forests is based on a combination of aerial and ground surveys and stream baiting (FHM projects). Current methods allow identification of *P. ramorum* by culturing and PCR analysis, but they do not identify clonal lineages or mating types, both of which are of great importance in terms of disease spread and potential damage to forest resources.

b. Significance/Impact of forest health issue. Sudden oak death is caused by *Phytophthora ramorum*, a pathogen of unknown origin and world-wide importance. It has been causing widespread mortality of coast live oak, tanoak, and black oak in California for nearly two decades, and now occurs in 14 counties there. In Oregon the disease currently is limited to a 210 mi² quarantine area, but it continues to spread despite an ongoing slow-the-spread program. In Europe, where the pathogen formerly occurred only in the nursery trade, it has spread to trees and forests. In the UK and Ireland thousands of acres of infested plantations containing Japanese larch, Douglas-fir, western hemlock, and Port-Orford-cedar have been cut to reduce the spread of the pathogen (Brasier et al., 2011). The pathogen has potential to spread throughout coastal forests of the US west coast and to cause considerable ecological and economic damage to the forestry and nursery industries (Vaclavik et al., 2010). On a local scale dead trees resulting from the disease increase the risk of wildfire and pose hazards associated with falling trees.

c. Scientific basis / likelihood of success. The investigators/cooperators have extensive experience in identifying and collecting *P. ramorum* isolates, conducting analyses of molecular markers, and interpreting genetic marker data. Cooperators have collaborated successfully on other projects.

d. Cost/Economic efficiency. Isolates (cultures) of *P. ramorum* are collected as part of the ongoing early detection program, so there will be no costs incurred for collecting field isolates from infected plants, soil sampling, or stream baiting. Isolates collected since 2001 are stored at Oregon State University and are available to the proposed study. The ARS lab (Grunwald) is already conducting molecular analyses, so there are no expected set-up costs.

e. Priority Issues. The proposed project addresses the following priority issues:

Fire risk and fuel loading. Extensive areas of tanoak mortality from sudden oak death are developing in Curry County, and likely will result in unprecedented fuel loads in coastal southwest Oregon. This can be mitigated to a degree by eradication of the pathogen from new infestations before the disease has a chance to intensify and spread. The proposed project will improve our ability to determine which new infestations are most important in terms of disease spread and subsequent mortality.

Ecological impacts of fire. The high fuel loads resulting from tanoak mortality will alter the ecological impacts of fire, potentially causing more frequent and intense fires (Valachovic et al., 2011). By identifying lineages of *P. ramorum* the proposed project will help establish treatment priorities by identifying source populations for novel infestations and pathways of infection which ultimately could be targets of management to reduce fire impact by removal of infectious/infected trees.

Invasive species. *P. ramorum* is one of the most important and damaging forest pathogens in the world. It is capable of changing ecosystems by killing several tree species. Sudden oak death has been shown to alter fire behavior in Coastal California. In Oregon, tanoak is at risk of being eliminated throughout most of its native range.

DESCRIPTION:

a. Background: At least four clonal lineages of *P. ramorum* (NA-1, NA-2, EU-1, EU-2) exist throughout the world in nurseries and natural environments, and these lineages differ in their ability to cause disease. Although several lineages have been found in west coast nurseries, only one lineage (NA-1) occurs in Curry county forests. The Curry county forest population of *P. ramorum* currently is reproducing asexually (clonally) because it consists of only one mating type. If another mating type is introduced (such as the EU-1 or EU-2 lineage), sexual reproduction could occur, potentially allowing the pathogen to adapt and become more damaging (Grunwald et al., 2012).

In June, 2012 a nursery in Curry County was found to be infested with *P. ramorum* and subsequent testing identified the clonal lineage as EU-1. Plants from this nursery had been sold prior to this detection, so there is a high risk that the EU-1 lineage has moved outside the nursery. The EU-1 lineage of *P. ramorum* is causing extensive mortality in hundreds of larch plantations in the United Kingdom and Ireland and also is damaging nearby Douglas-fir and hemlock.

Lineage and mating type can only be determined by molecular/phenotypic assays that are not part of our regular detection program. The current program only determines whether or not the pathogen recovered from field samples is *P. ramorum*. Analyzing genetic markers (microsatellites or SSRs) present in samples collected during our aerial and ground surveys will allow us to identify high-priority infestations for eradication (sites with new lineages), understand spread of the pathogen, and identify pathways of disease spread.

b. Methods:

Microsatellite analysis. Microsatellite genotypes will be determined using a range of microsatellite loci characterizing all extant clonal lineages as described previously (Goss et al., 2011; Prospero et al., 2009). Allele size in bp will be determined on a capillary sequencer available in the Grünwald lab for this purpose (ABI 3100 Avant). Electropherograms will be analyzed using GeneMapper software (version 3.7 Applied Biosystems) to size PCR fragments.

Data analysis. Standard population genetic analysis based on genotype and allele frequencies will be conducted. In addition, powerful new methods for determining population patterning over time and space have recently been developed and as a result a more detailed description of the dynamic processes leading to population divergence can now be inferred (Grünwald and Goss, 2011). For *P. ramorum*, determining migration rates among populations and linking source and sink populations will be critical in understanding the movement of the pathogen and establishment of new populations. We have applied these techniques recently to infer that the EU1 clonal lineage migrated from Europe to North America (Goss et al., 2011).

c. Products:

New strains and lineages of *P. ramorum* continue to emerge. For example, in 2011 a novel clonal lineage now named EU-2 was detected in Northern Ireland and since in Western Scotland. Based on microsatellite analysis it has become clear that clonal lineages continue to evolve at a rapid pace. Microsatellite markers are thus very powerful in providing insights into contemporary migrations of *P. ramorum* and identifying novel introductions. Knowledge of migration patterns and novel introductions help management of epidemics.

d. Schedule of Activities:

Year 1: Update and harmonize information in forest and nursery databases for *P. ramorum* genotypes. Genotype any new and some existing populations of *P. ramorum*.

Years 2 & 3: Genotype any new and some existing populations of *P. ramorum*.

e. Progress/Accomplishments:

Detection surveys and isolate collections continue as scheduled. Existing forest isolates from Curry County have been genotyped and catalogued. This includes 207 and 144 forest collections from 2012 and 2013, respectively. The SSR database has been updated. To date, no isolates of the European lineages (EU-1 or EU-2) have been recovered from Curry County forests.

The disease continues to intensify within the generally Infested Area (GIA) and is slowly expanding elsewhere within the quarantine area. Thus far in 2013, ten new outlier or leading edge infestations have been discovered; all are within the quarantine boundary but outside of the GIA. Numerous isolates have been collected from these sites. Two of these sites were located more than one mile from the nearest known infestation.

f. Relevant Citations:

Brasier, C. and Webber, J. (2010). Plant pathology: sudden larch death. *Nature* 466, 824–825.

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

Goss, E.M., Larsen, M, Vercauteren, A., Werres, S., Heungens, K., & Grünwald, N. (2011). *Phytophthora ramorum* in Canada: evidence for migration within North America and from Europe. *Phytopathology* 101:166-171.

Grünwald, N. J., Garbelotto, M., Goss, E. M., Heungens, K., and Prospero, S. (2012). Emergence of the sudden oak death pathogen *Phytophthora ramorum*. *Trends in Microbiology* 20:131-138.

Grünwald, N. J. and Goss, E. M. (2011). Evolutionary and population genetics of exotic and re-emerging pathogens: traditional and novel tools and approaches. *Ann. Rev. Phytopathology* 49: 249-267.

Kanaskie, A., Hansen, E., Goheen, E.M., Osterbauer, N., McWilliams, M., Laine, J., Thompson, M., Savona, S., Timeus, H., Woosley, B, Sutton, W., Reeser, P., Schultz, R., & Hilburn, D. (2011). Progress of the *Phytophthora ramorum* eradication programme in southwest Oregon Forests, 2001-2009. *New Zealand Journal of Forestry Science* 41S:177-187.

Prospero, S., Grünwald, N. J., Winton, L. M., & 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.

Vaclavik, T., Kanaskie, A., Hansen, E.M., Ohmann, J.L., & Meentemeyer, R.K. (2010). Predicting potential and actual distribution of sudden oak death in Oregon: Prioritizing landscape contexts for early detection and eradication of disease outbreaks. *Forest Ecology and Management* 260(6):1026-1035.

Valachovic, Y.S.; Lee, C.A.; Scanlon, H.; Varner, J.M.; Glebocki, R.; Graham, B.D.; and Rizzo, D.M. (2011). Sudden oak death-caused changes to surface fuel loading and potential fire behavior in Douglas-fir-tanoak forests. *Forest Ecology and Management* 261 (11): 1973-1986.

COSTS / BUDGET: If the grant is awarded, funds will be transferred to USDA-ARS through an interagency agreement.

Year		Item	Requested FHM EM Funding	Other-Source Funding	Source
FFY 2013	Administration	Salary	\$20,000	\$25,000	ODF, OSU, ODA: isolate collection and preparation
		Salary		\$5,000	ARS, technical assistance
		Overhead	\$4,000		
		Travel	\$1,000		
	Procurements	Contracting			
		Equipment			
		Supplies	\$5,000		
	Total (2013)		\$30,000	\$30,000	

FFY 2014			\$15,000	\$12,000	ODF, OSU, ODA: Detection surveys, isolate collection and preparation
				\$3,000	ARS, technical assistance
FFY 2015			\$15,000	\$12,000	ODF, OSU, ODA: Detection surveys, isolate collection and preparation
				\$3,000	ARS, technical assistance