

Tracking Populations and New Infections of *Phytophthora ramorum* in Southern Oregon Forests¹

Jennifer Britt,² Simone Prospero,³ Niklaus Grünwald,⁴ Alan Kanaskie,⁵ and Everett Hansen²

Abstract

Since the discovery of *Phytophthora ramorum* in southern Oregon forests in 2001, newly infested areas are located each year. We tracked the spread and dispersal using DNA fingerprinting. While among site genetic variance was low, we did find changes in genotype presence and frequency at the site level. These genotypic differences allowed us to map the spread of some individual genotypes demonstrating long and short distance dispersal of *P. ramorum* in Oregon Forests.

Previous genetic work has shown the Oregon forest population belongs to the North American one clonal lineage, it is distinct from the Oregon nursery populations, and has low genetic diversity within Oregon forests (Ivors and others 2006, Prospero and others 2007). Although the molecular diversity is low, we can use DNA markers to track the spread of *P. ramorum* in Oregon forests and give insight into how the pathogen is spreading.

Using microsatellite markers, our study aims to answer the following:

Do the new infections represent novel introductions or come from previously infested areas?

Does the genetic infection pattern suggest mode(s) dispersal?

We collected samples from a variety of hosts, including: tanoak (*Lithocarpus densiflorus*), rhododendron (*Rhododendron* sp.), evergreen huckleberry (*Vaccinium ovatum*), Oregon myrtle (*Umbellularia californica*), and poison oak (*Toxicodendron diversilobum*), and from streams and soil from 2001 through 2008. We plated the samples onto *Phytophthora* selective CARP medium in the field and/or laboratory and grown cultures out in the laboratory for identification (Hansen and others 2005). The southern Oregon range of *P. ramorum* was delineated into sites based on stream watershed, topography, and in some cases, distance. We genotyped a total of 1925 individual isolates at 5 microsatellite loci PrMS39, PrMS43, and PrMS45 (Prospero and others 2004), and PrM82 (Ivors and others 2006) on an ABI 3100 DNA sequencer. All isolates of any genotype but the most common, and approximately 25 percent of the most common isolates, were re-extracted and/or re-genotyped. Data was analyzed using the software package GenAlEx6 (Peakall and Smouse 2006).

We identified 68 novel multilocus genotypes (MGs) with 10 to 35 MGs found in each year. While the majority of MGs were present in very low numbers (< 1 percent), one MG was

¹ A version of this paper was presented at the Fourth Sudden Oak Death Science Symposium, June 15-18, 2009, Santa Cruz, California.

² Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97333.

³ Ecological Genetics and Evolution, Swiss Federal Institute for Forest, Zürcherstrasse 111, CH-8903 Birmensdorf.

⁴ Horticultural Crops Research Laboratory, USDA ARS, Corvallis, OR 97330.

⁵ Oregon Department of Forestry, Forest Health Management, 2600 State Street, Salem, OR 97310.

Corresponding author: brittje@science.oregonstate.edu.

dominant in all years representing 35 to 65 percent of isolates. Six genotypes were present in all 8 years while 31 genotypes were present in only 1 year. We found no MGs matching any found in Oregon nurseries or in California.

A population assignment test, that uses expected genotype frequencies across loci to assign individuals to their population or a different population, revealed very little population structure among designated populations. Fifteen percent of individuals were assigned to their “self” population while 85 percent were assigned to an “other” population.

Despite low overall genetic variability in *P. ramorum* among sites in southern Oregon, differences in MGs among individuals are informative in tracking the spread through the forest. Figure 1 shows the different genotypic frequencies at the 35 delineated sites.

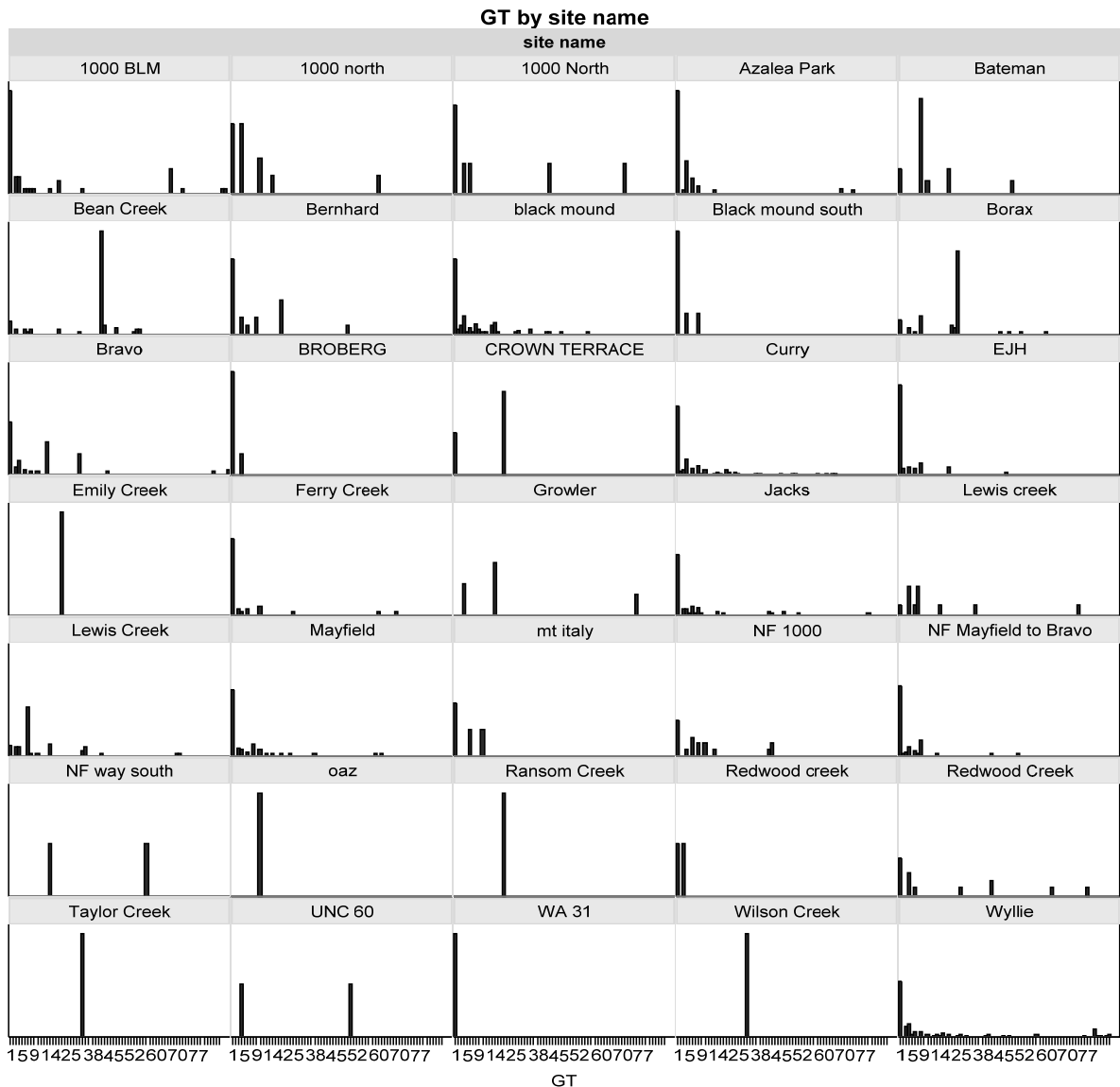


Figure 1—Multilocus genotype frequencies at 35 sites in southern Oregon forests.

What is most interesting is the dominance of different genotypes among sites. For example, MG 41 is present in over 50 percent of samples from Bean Creek, but is absent in all but a few samples at two other nearby sites. We can also see that the most common genotypes are

present at most of the sites. When this same information is mapped among sites, dispersal distances can be determined. Site level population multilocus genotypic differences supports the theory of wind and splash dispersal events where individual genotype(s) are dispersed by wind to start a new subpopulation and spread in small areas by splash dispersal.

In summary, DNA fingerprinting allows us to track new and existing *P. ramorum* infections in Oregon forests, with no new infections from California, Washington, or nurseries detected since 2008. We are also able to map how the pathogen is spreading through the Oregon forest, suggesting long distance dispersal by wind and short distance dispersal by splash events.

Our current efforts are aimed at examining the fitness of the most common genotypes in an effort to parse apart whether the dominance of one genotype is based on a founder event or the advanced fitness of that particular genotype.

Acknowledgments

We would like to thank the U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station for funding; the Oregon Department of Forestry for collections and field work; and Paul Reeser and Wendy Sutton for lab help.

Literature Cited

Prospero, S.; Hansen, E.M.; Grunwald, J. and Winton, L.M. 2007. Population dynamics of the sudden oak death pathogen *Phytophthora ramorum* in Oregon from 2001 to 2004. *Molecular Ecology*. 16: 2958–2973.

Ivors, K.L.; Garbelotto, M.; De Vries, I.; Ruyter-Spira, C.; Te Hekkert, B.; Rosenzweig, N. and Bonants, P. 2006. Microsatellite markers identify three lineages of *Phytophthora ramorum* in US nurseries, yet single lineages in US forest and European nursery populations. *Molecular Ecology*. 15: 1493–1505.

Peakall, R. and Smouse, P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*. 6: 288–295.

Prospero, S.; Black, J.A. and Winton, L.M. 2004. Isolation and characterization of microsatellite markers in *Phytophthora ramorum*, the causal agent of sudden oak death. *Molecular Ecology Notes*. 4: 672–674.

Hansen, E.M.; Parke, J.L. and Sutton, W. 2005. Susceptibility of Oregon forest trees and shrubs to *Phytophthora ramorum*: a comparison of artificial inoculation and natural infection. *Plant Disease*. 89: 63–70.