Dynamics of Aerial and Terrestrial Populations of *Phytophthora ramorum* in a California Watershed Under Different Climatic Conditions[1]

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Abstract

We present a study of the epidemiology of sudden oak death (SOD) in California within a watershed based on temporally and spatially replicated surveys of symptoms, viability of the pathogen from symptomatic leaves, and genetic analyses using polymorphic SSR markers.

Phytophthora ramorum is sensitive to climate; its optimal growth and transmission conditions are in the spring with warm rainy weather, and although it can survive and persist in harsh conditions, its capacity for transmission and dispersal are significantly reduced in drought conditions. Its main method of transmission is via rainsplash. The San Francisco Bay Area experienced a period of drought for several years prior to, and including, 2009, where conditions for *P. ramorum* were suboptimal and SOD outbreaks were notably fewer. However, 2010 saw a return to wetter conditions. We studied the population dynamics of *P. ramorum* resident in two different substrates (leaves and soil) during that climatic transition to study the effects on diversity and isolation success. Population genetics have been used to reconstruct the global history and migration of the pathogen to understand its origins and emergence as a significant pathogen in North America and Europe. Our study is one of the first to address the population dynamics of *P. ramorum* at a local micro-evolutionary scale and to compare the populations in different substrates.

Intense sampling of soil and leaf populations was carried out over a period of 2 years which spanned a climatic transition from drought in 2009 to a wetter climate in 2010. The survey was conducted in the San Francisco Public Utilities Commission watershed district, near San Mateo, California. The area has been infested for over 10 years and is subject to minimal management, so it is relatively undisturbed. Six survey plots were set up in two drainages within the watershed in areas of coast live oak (*Quercus agrifolia* Née), tanoak (*Notholithocarpus densiflorus* Manos, Cannon & S.H. Oh) and California bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.). Leaves were sampled five times during the 2-year study period for leaves: Early (March/April), Peak (June), and Late (September) 2009; and Early and Peak 2010. Soil was sampled twice during Peak time each year. Each plot consisted of six 100 m transects radiating out from a central point. Symptomatic leaves were sampled from the canopy of California bay laurel trees at 10 m intervals along each transect. Simultaneously, soil was sampled from three areas around the base of the tree and amalgamated. Leaves were plated onto PARP selective growth medium (pimaricin; 400µl/L, ampicillin; 250 mg/L, rifampicin; 10 mg/L, PCNB; 25 mg/L), incubated, and *P. ramorum* growth identified and subcultured. Soil samples were flooded with dH_2O and baited with leaf discs taken from uninfected *Rhododendron* var Cunningham's White leaves for 5 to 7 days, then leaf discs plated onto PARP+H (as PARP above but with addition of 25 mg/L hymexazol). *Phytophthora ramorum* growth was subcultured onto PARP.

Mycelial cultures were grown in liquid pea broth and DNA extracted with NaOH extraction (Wang et al. 1993). DNA was amplified using fluorophore labeled primers for six different microsatellite loci: MS18, MS64 (Ivors et al. 2006), MS38, MS43, MS45 (Prospero et al. 2007), MSILVO145 (Vercauteren et al. 2010). Multilocus genotypes (MLG) were assigned using Gimlet software. Genetic diversity indices were calculated: Stoddart & Taylor's G; G^ percentage of maximum diversity i.e., G/N, where N is sample size; R, clonal genotype diversity where $R=(G-1)/(N-1)$ where G is the number of MLGs present in a sample and N is the sample size. Bruvo distances were calculated between MLGs and populations analyzed for genetic structuring by Analysis of Molecular Variance (AMOVA) analysis using Arlequin v3.1.5.2.

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Symptoms of SOD on leaves of the transmissive host, California bay laurel, increased significantly from 15 to 39 percent in six survey plots between dry and wet conditions, while levels of identical symptoms caused by other foliar pathogens were highest (69 percent) in dry conditions. This suggests that *P. ramorum* and other pathogens, while occupying the same niche, are favored by different climatic conditions. *Phytophthora ramorum* isolation success from leaves was lowest during the cold dry fall/Late season in 2009, and the greatest number of isolations was made during the Peak 2010 when conditions were rainy and warm. Isolation from soil was very similar in both years, with isolation success decreasing slightly in 2010. The soilborne population seems less labile with respect to climate; the soil environment probably provides a better buffer to climatic change than the tree canopy.

The populations sampled were very diverse. In leaves, 22 MLGs were detected in Peak 2009, and the number detected more than doubled to 49 MLGs in Peak 2010. In soil, the number was more consistent between years; 20 MLGs were detected in 2009, 23 MLGs in 2010. Many of the genotypes detected were singletons (i.e., only found in one individual). Diversity indices were calculated using the multilocus genotypes of isolates from these times. Using Stoddart and Taylor's *G* ,which is an absolute measure, the diversity increased throughout the sampling period, tracking the increasing numbers of MLGs detected, and was greatest in Peak 2010 for both substrates with diversity greater in leaves than in soil (Leaf: G=14.687, Soil: G=12.27) (fig. 1). Indices *G^* and *R*, which take sample size into account, followed very similar patterns to each other and showed an almost inverse relationship to *G*. According to *G^* and *R*, diversity was lowest during the Peaks of 2009 and 2010 as compared with other sampling seasons. These low values for diversity indices at the times of year when the most isolations and most MLGs were detected indicates that the populations were dominated by a few highly abundant genotypes. This effect was strongest in 2010, when the lowest values for *G^* and *R* were recorded, coinciding with when the climate had transitioned to wetter conditions. In these favorable conditions for *P. ramorum* growth and sporulation, there is more inoculum present leading to greater competition for space and resources between individuals. It may be that certain individuals have some selective advantage and those phenotypes are selected for and become dominant within the population. Although microsatellite markers are selectively neutral and do not confer any advantage on an individual, the advantageous phenotypes may carry the MLGs to dominance by association.

Analysis of genetic structuring of populations showed that there was no structure between the two drainages, but there was structure within the drainages between the individual plots. This is likely to have arisen due to local evolution of genotypes from small founding populations and restricted movement of the pathogen, especially in the drought years. This structuring was more pronounced in leaf populations in 2010, probably as a reflection of the more favorable conditions and proliferation of the pathogen-enhancing founder effects. There was significant structuring between soil and foliar populations, but soil and leaf genotypes were generally intermixed and closely related, indicating they are part of the same source population. It may be that there is a different ability of genotypes to adapt to different substrates. Very few genotypes were shared between substrates, and where they did overlap, the relative proportions in each substrate were significantly different. Finally, leaf populations were similar between sampling times, but soil populations showed some structure, suggesting that there is turnover between years in the soil population. Rarefaction was used to ensure that sample sizes were sufficient to capture a good representation of the diversity present to ensure that turnover was not an artifact of insufficient sampling of a very diverse population.

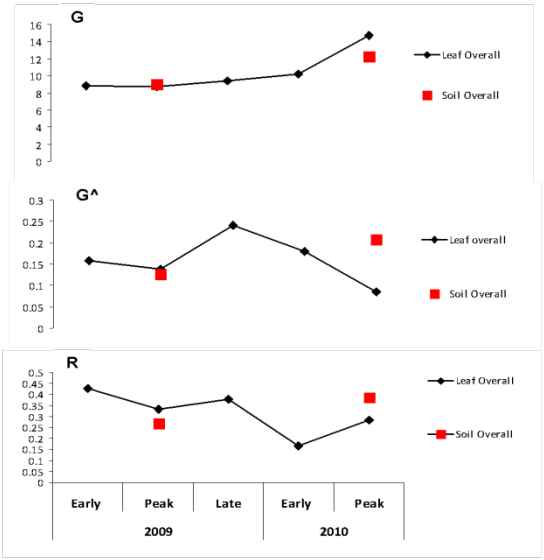

Figure 1—Genetic diversity indices for soil and leaf populations of *Phytophthora ramorum* sampled in 2009 and 2010.

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