Microsatellite Analysis of the EU1 Lineage of *Phytophthora ramorum* in Washington State Nurseries, Landscapes, and Waterways[1]

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Abstract

Microsatellite analysis initially identified genetic variations within the NA1 clonal lineage of *Phytophthora ramorum*; however, in Washington nurseries, the genetic population of *P. ramorum* has shifted and is now dominated by two other lineages, NA2 and EU1. In this study, recently identified markers that are more variable, and therefore more informative for the EU1 lineage, were used to reveal genetic diversity within the Washington *P. ramorum* EU1 population. Data from genotyping of DNA samples provided by the Washington State Department of Agriculture (WSDA) was combined with new detailed genotype data of DNA from isolates collected by Washington State University (WSU) representatives to examine the structure of the Washington nursery EU1 population.

Fifty-eight *P. ramorum* isolates in WSU's Master Collection were previously determined to be within the EU1 lineage. Forty-five of the cultures were isolated from *Rhododendron*, six from *Viburnum*, five from *Kalmia*, and two from soil baits. Two of the isolates, one each from *Rhododendron* and *Viburnum*, were from Oregon; the remaining 56 were from Washington nurseries or trace-forward sites within Washington. The samples were analyzed with 12 previously described microsatellite (SSR) loci. One hundred nineteen EU1 DNA samples from infected plant material collected at Washington nurseries by WSDA staff were included in some analyses.

One marker locus (Ivors82) was excluded from analysis due to hypervariability in several samples between labs. Analysis with the R population genetic analysis program *poppr* showed that seven of the 11 remaining loci are uninformative within this sample set, leaving the following four informative loci: PrMS45, Ivors64, ILVOPrMS131, and ILVOPrMS145c. A total of 27 multi-locus genotype (MLG) groups were identified, revealing a great deal of variability that was not detected previously. The locus with the highest allelic diversity, ILVOPrMS131, had 16 alleles and 24 allele combinations (genotypes) in 58 isolates. Calculations using Bruvo's genetic distance inferred four major groupings containing six population clusters

Among the Washington nurseries and trace-forward sites, the highest genetic diversity was found at a nursery referred to as Nursery #43, with five samples collected in the same year having five unique genotypes. This is possibly the result of a long-term infestation, multiple introductions from other nurseries, or less likely, a single introduction event with high genetic diversity. The lowest diversity was found in the 18 isolates collected over 4 years from Nursery #41 and its trace-forward sites.

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