An Update on Microsatellite Genotype Information of *Phytophthora ramorum* in Washington State Nurseries[1]

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Abstract

Phytophthora ramorum was first detected in a Washington nursery in 2003 and has since been positively identified in 46 nurseries, three non-nursery water sites, and three landscape sites. Thirteen nurseries have tested positive for 2 consecutive years and four nurseries have been positive for 3 consecutive years, despite the completion of the U.S. Department of Agriculture Animal and Plant Health Inspection Service (APHIS) Confirmed Nursery Protocol (CNP), which is intended to eradicate this pathogen from positive nurseries. Trace-back inspections to wholesale nurseries have failed to confirm the origin of infested plant material at some nurseries and thus it is unclear if the pathogen is being reintroduced into these repeat nurseries each year or if it is persisting at the nurseries from one year to the next.

In an effort to better understand the population structure of *P. ramorum* in Washington, 328 isolates or DNA samples were collected in cooperation with the Washington State Department of Agriculture (WSDA) over 5 years (2005 to 2009) from 30 Washington nurseries, three non-nursery water sites, and three landscape sites, and genotyped using eight previously described microsatellite markers.

All three previously described lineages (EU1, NA1, and NA2) were detected in each of the 5 years. In this population, the EU1 lineage is represented by one genotype, the NA2 lineage is represented by one genotype, and the NA1 lineage is represented by 45 genotypes. The NA1 lineage was the most common, occurring in 25 nurseries, three non-nursery water sites, and one landscape site. The NA2 lineage was detected at 11 nurseries and one non-nursery water site, while the EU1 lineage was detected at seven nurseries and two landscape sites. The occurrence of the EU1 lineage in Washington has increased in frequency over the past 4 years while the overall number of sites and isolates has declined. At one nursery in 2007, the NA1 and EU1 lineages were isolated from different branches on the same rhododendron plant and at a different nursery in 2008 in the same soil bait. Although no genotype detected to date possesses a hybrid of alleles from both the European (EU1) and North American (NA1 and NA2) lineages, the combined presence of these lineages poses an increased risk to Washington because of the potential for sexual recombination.

The CNP appears to be effective in eradicating the pathogen from infested nurseries in some instances. Twenty-nine of the 45 positive nurseries were negative for *P. ramorum* in the year following completion of the CNP. On the other hand, 17 nurseries were positive for 2 or more years in a row. At only two repeat positive nurseries did a new lineage appear in the second

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positive year that was not present in the nursery during the first positive year; however, our genotype sample sizes are small. Thus it is unclear if inoculum was persisting from year to year in these nurseries or if the same genotypes were reintroduced in subsequent years.

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