

Potential for Sexual Reproduction of *Phytophthora ramorum* in Washington State Nurseries

Matteo Garbelotto, Kelly Ivors, Daniel Huberli, Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA 94720, (510) 643-6412, matteo@nature.berkeley.edu; Peter Bonants, Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands; and Art Wagner, Washington State Department of Agriculture, Plant Protection Division, Olympia, WA 98501

In 2003, isolates belonging to the A1 mating type were reported from commercial nurseries in Oregon State. Soon after, we reported the presence of both mating types of *P. ramorum* in nurseries in Washington State. AFLP, microsatellite, and RFLP of the COX region all indicated the A1 isolates belonged to the European lineage of *P. ramorum*. In 2003, 33% of the isolates analyzed belonged to European lineage, while the rest belonged to the North American lineage. Isolates belonging to the two lineages were found in the same plant block, and even on adjacent plants. By using tester strains (A2 from California and A1 from Oregon nurseries) we confirmed that all North American isolates belonged to the A2 mating type, and all of the European isolates belonged to the A1 mating type. The coexistence of both mating types on adjacent plants increases the chances for sexual recombination between these two genetically extremely divergent lineages. Our crosses with tester strains were 100% successful, but a very high proportion of oospores appeared to be aborted, suggesting the mating system of *P. ramorum* is not perfectly functional, potentially because of the long isolation of the two mating types. Nonetheless, a significant number of oospores appeared to be perfectly viable. In 2004, the survey of isolates from Washington state nurseries continued. COX RFLPs and morphology of the colony (appressed for the US and fluffy for the EU isolates) indicated that 84 appressed colonies had the EU RFLP pattern, while only one isolate had fluffy colony morphology and had a RFLP pattern matching that of the EU lineage. Despite the sharp decrease of A1 isolates in 2004, we encountered one isolate that had fluffy colony morphology and RFLP pattern of the USA isolates. Out of 12 microsatellite loci analyzed for this isolate, two had alleles exclusively found in the US, two had alleles exclusively found in Europe, one had one allele that is shared by both lineages, and 7 had novel alleles. AFLP analysis showed this isolate has bands typical of the US lineage, bands typical of the EU lineage, and a significant number of new bands. This isolate is likely to be either a recombinant between A1 and A2 or an isolate representative of a third yet unknown lineage. Mating and phenotype of this isolate will be discussed, including implications for the evolution of *P. ramorum*.