Population Structure of *Phytophthora ramorum* in Oregon [1]

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Abstract

Phytophthora ramorum is infecting plants in Oregon forests and nurseries. In this study, we analyzed the population structure of the *P. ramorum* in Oregon from 2001 to 2004 using microsatellites. The *P. ramorum* population in Oregon is characterized by low genetic diversity, significant genetic differences between nursery and forest populations, and no evidence of sexual recombination. The forest population is dominated by a single multilocus genotype in all four years, indicating the persistence of pathogen inoculum. In nurseries, identical genotypes were not recovered during both sampling years (2003 and 2004), suggesting pathogen eradication was effective. This research highlights the continued importance of sanitation and quarantine in nursery production to prevent further introduction and spread of *P. ramorum*.

Key words: *Phytophthora ramorum*, population genetics, microsatellites.

Introduction

P. ramorum was first detected in Oregon forests in 2001 and nurseries in 2003. Quarantine and eradication efforts are currently in operation. Nevertheless, newly infected plants are detected every year. In order to determine the persistence and spread of *P. ramorum* in Oregon forests and nurseries, we designed a study using population genetic techniques. We investigated the following questions: (1) what is the population structure of *P. ramorum* in Oregon, (2) is sexual recombination taking place, (3) and are the eradication and quarantine efforts working?

Materials and Methods

A total of 323 isolates of *P. ramorum* were collected from 2001 to 2004; 272 were recovered from the infested forest and 51 were obtained from nurseries. About 60 percent of the forest isolates were collected from infected trees or shrubs, thirty isolates were obtained from baits in seven streams and 29 isolates were obtained from soil samples. The 51 nursery isolates originated from infected plants grown in 15 nurseries.

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[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

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The 2001 to 2004 isolates of *P. ramorum* were screened for alleles at 10 microsatellite loci. The di-nucleotide and tri-nucleotide loci were previously isolated from an enriched genomic library as described by Prospero and others (2004). Four additional loci were identified by screening the genome of *P. ramorum* (available online at: http://genome.jgi-psf.org/) for common tetra-nucleotide repeats.

Results and Discussion

This study suggests that the nursery and forest populations of *P. ramorum* in Oregon are distinct. A total of 33 multilocus genotypes were identified among isolates collected from 2001 to 2004; 22 genotypes were only recovered from the infested forest, nine genotypes were only detected in nurseries, and two genotypes were observed both in the infested forest and in nurseries. Genetic variation within populations, estimated with Stoddart and Taylor's G index, was very similar for forest populations of *P. ramorum* from all four sampling years. Support for similarities in the *G* values among populations was tested with bootstrapping at a confidence level of 90 percent. Including the nursery isolates in the 2003 and 2004 populations of *P. ramorum* caused an increase in the genetic variation both in 2003 and 2004. Genetic variation among populations was estimated with Weir & Cockerham's (1984) coefficient of differentiation θ and distance trees based on Nei's (1972) genetic distance. No differences were found among the four annual forest populations ($\theta = 0.0006$, $P = 0.302$). The forest and nursery populations were significantly different ($\theta = 0.316$, $P < 0.001$) as were the four annual *P. ramorum* populations when forest and nursery isolates were combined ($\theta = 0.0283$, $P < 0.001$). The nursery populations in 2003 and in 2004 were also significantly different ($\theta = 0.160$, $P < 0.001$). The trees based on Nei's genetic distance showed that the four annual forest populations of *P. ramorum* were genetically very close (0.003, fig. 1A). A considerably larger genetic distance was observed between the forest population and the nursery population (0.235, fig. 1C). Comparative trees derived from clone-corrected data sets showed qualitatively similar results (0.015 and 0.160 fig. 1B and D respectively).

Several results indicate clonal reproduction in the *P. ramorum* population in Oregon. First, there is low genotypic diversity, with most isolates belonging to a dominant genotype. Second, the observed heterozygosity is higher than that expected under Hardy-Weinberg equilibrium, resulting in a negative inbreeding coefficient (F_{IS}). In diploid organisms, extreme clonality often results in considerable heterozygote excesses (Balloux and others 2003). Third, the index of association indicated the presence of significant linkage disequilibrium in all populations when a single representative of the European genotype was added to the data set (Dobrowolski and others 2003).

Our study reveals that *P. ramorum* eradication efforts in nurseries are working. The nursery population of *P. ramorum* was not dominated by a single multilocus genotype. The most common genotype comprised 25.5 percent of the isolates and was found in six out of 15 nurseries. Each of the other 10 genotypes had a frequency of 2 to 20 percent. None of the 11 nursery genotypes found in 2003 and 2004 was detected in both sampling years. This suggests effective eradication of nursery infestations in 2003, followed by new introductions from different sources in 2004.

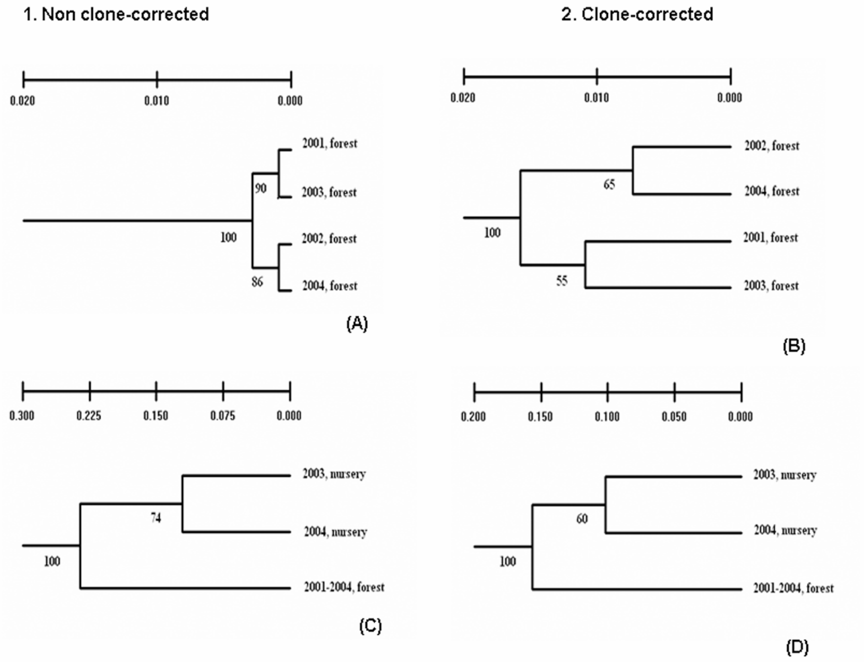

Figure 1—Phenogram constructed using the unweighted pair-group method of averages (UPGMA) algorithm based on the Nei's (1972) genetic distance of populations. Statistical support for branches was obtained using 1000 bootstrapped samples of the data set. The numbered bars show the distance. In A and B, only forest isolates are included in the analysis. In C and D, forest and nursery isolates were included in the analysis.

Our study also suggests that quarantine efforts in the Oregon forest are highly effective at stopping the introduction of new infections to the forest. In all four sampling years, the forest population was dominated by a single multilocus genotype which persisted over all years and was found in all main infection centers. All but two other genotypes occurred at less than 5 percent frequency. Four genotypes were found in all four sampling years, whereas 12 genotypes were only detected in one particular year. This is suggestive of a likely single introduction with the persistence of an inoculum source responsible for new infections as it spreads through the forest. Destruction of all potential hosts growing around a symptomatic plant clearly reduces the inoculum levels and the spread of the pathogen, but has not completely prevented continuing infections.

The presence of different *P. ramorum* genotypes in Oregon forests and nurseries emphasizes the importance of prevention and sanitation practices in nurseries in order to reduce the risk of introducing new populations of *P. ramorum* into forest ecosystems.

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