

# Genotyping *Phytophthora ramorum* isolates from US nurseries using PCR-RFLP and microsatellite analyses

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## Introduction

The risk of introducing the important plant pathogen *Phytophthora ramorum* outside of its present natural range is of extreme concern; however the detection of *P. ramorum* in U.S. nurseries is increasing.

USDA APHIS has confirmed the presence of *P. ramorum* in nurseries in twenty-one states in the US that received plants from an infested southern California nursery.

Although it remains unknown whether these new introductions will lead to an outbreak of sudden oak death (SOD) in the affected states, many potential susceptible hosts of *P. ramorum* are widely distributed in US forest ecosystems, and many of the affected states have climatic conditions conducive for SOD.

## Materials and methods

As a part of an effort to genotype new nursery infestations, we characterized nineteen *P. ramorum* isolates from thirteen states with *cox1* RFLP banding patterns (Kroon et al., 2004) and microsatellite analyses.

### PCR-RFLP

*Cox1* PCR → *Apo1* Restriction enzyme digestion → Gel separation and fragment visualization

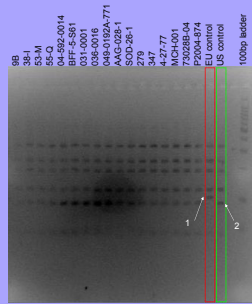
### Microsatellite

PCR with labeled primers → ABI 3100 → Fragment analysis using Genescan software

## Results

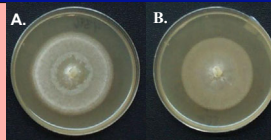
To date, almost all isolates from Europe are mating type A1, while those from the US are A2. The presence of both mating types in the same location could lead to a population capable of sexual recombination.

*Cox1* PCR-RFLP is a method originally developed by Kroon et al. (2004) and can be used to distinguish isolates of *P. ramorum* from Europe and those from the US. European isolates have a cytosine residue on position 773 of the 972-bp *Cox1* amplicon, while US isolates have a thymine residue at this position.



## PCR-RFLP

**Fig. 1.** Restriction fragment length polymorphism pattern for 972-bp amplicon of the Cytochrome c oxidase subunit I gene for *Phytophthora ramorum* isolates collected from infested nurseries. Arrows indicate (1) European control fragment of 96 bp, and (2) US control fragment of 84 bp. Isolates in lanes 1-17 have a 'US' type RFLP pattern.



**Fig. 2.** *P. ramorum* isolates growing on V8 agar. (A) EU type with characteristic aerial mycelium, and (B) US type with appressed mycelium.

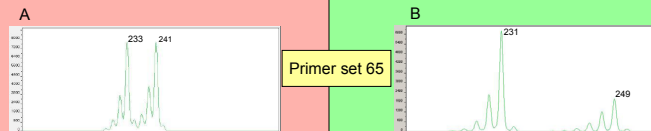
Microsatellites are tandemly repeated sequences. Di-, tri- and tetra-nucleotide repeats are the types mostly commonly utilized. Alleles at a given locus are identified following a specific PCR protocol. The ABI 3100 sequencing machine, along with Genescan software are used for generating and analyzing microsatellite fragment data.

14 informative microsatellite loci (K. Ivors, in publication) were also amplified to investigate the genetic variability of these important nursery isolates.

Europe

United States

## Microsatellite Analyses



**Fig. 3.** Generated Genescan data displaying 1 of 14 microsatellite loci (using primer set 65). (A) Europe isolates showing peaks at 233 and 241 bp, and (B) US isolates showing peaks at 231 and 249 bp.

**TABLE 1.** Nursery isolates of *Phytophthora ramorum* used in the study; designations, location, and host plant from which isolate was recovered.

ID	State	Host	PCR-RFLP		Microsatellite	
			EU type	US type	EU type	US type
9B	Georgia	Camellia sasanqua var. Yuletide		X		X
3B-1	Georgia	Camellia japonica var. Winters Fire/Ice Angels		X		X
53-M	Georgia	Camellia sasanqua		X		X
55-Q	Georgia	Camellia japonica var. Debbie		X		X
04-592-0014	Colorado	Camellia japonica var. Eleanor's Spieldor		X		X
BFF-6-SR1	Tennessee	Camellia sp.		X		X
031-0001	N. Carolina	Camellia japonica var. Pearl Maxwell		X		X
03B-0016	N. Carolina	Camellia japonica var. Mathotlanaa Supreme		X		X
04B-0192A-771	Texas	Viburnum tinus var. compactum		X		X
AAG-028-1	New Mexico	Camellia japonica		X		X
SOD-26-1	Louisiana	Viburnum tinus		X		X
279	Alabama	Camellia sp. var. April Remembered		X		X
347	Alabama	Camellia sp. var. Nicotia Gem		X		X
4-27-77	Arkansas	Camellia sp. var. April Remembered		X		X
MCH-001	Virginia	Camellia sp.		X		X
73028B-04	Pennsylvania	Camellia sp. (borsari)		X		X
P2004-874	Florida	Camellia japonica		X		X
1314176-9	California	Camellia sp.		X		X
1314176-13	California	Camellia sp.		X		X

**TABLE 2.** Results from (A) PCR-RFLP and (B) Microsatellite analyses

Isolates	A PCR-RFLP		B Microsatellite	
	EU type	US type	EU type	US type
9B		X		X
3B-1		X		X
53-M		X		X
55-Q		X		X
04-592-0014		X		X
BFF-6-SR1		X		X
031-0001		X		X
03B-0016		X		X
04B-0192A-771		X		X
AAG-028-1		X		X
SOD-26-1		X		X
279		X		X
347		X		X
4-27-77		X		X
MCH-001		X		X
73028B-04		X		X
P2004-874		X		X
1314176-9		X		X
1314176-13		X		X

## Conclusions

All 19 trace-back isolates originating from a southern CA nursery infestation produced the typical "US" RFLP pattern and showed no variation among the 14 microsatellite loci analyzed. Results indicate that these isolates consist of a single genotype, identical to *P. ramorum* isolates established in the wild in California and southern Oregon.

This discovery is in contrast with findings from other nursery infestations in Oregon and Washington, where both the European and US genotypes have been identified in infested blocks of host plants.

## Literature cited

Kroon, L.P.N.M., Verstappen, E.C.P., Kox, L.F.F., and Bonants, P.J.M. 2004. A rapid diagnostic test to distinguish between American and European populations of *Phytophthora ramorum*. *Phytopathology* 94, 613-620.

K. L. Ivors, D. Huberli, and M. Garbelotto. Detection of Both Mating Types of *Phytophthora ramorum* in the Same Location in North America. unpublished

## Acknowledgments

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## For further information

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