

Phenotypic Variation Among *Phytophthora ramorum* Isolates From California and Oregon¹

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Introduction

To manage and control *Phytophthora ramorum* successfully, it is important to know the amount of phenotypic variation within a given pathogen population. Because the pathogen has only recently been described, there are few studies on morphological and pathological variation of isolates from the United States. One study has compared growth rate on agar, aggressiveness to *Quercus rubra* (Eastern American red oak), and developmental stability of European and U.S. isolates. In this study, the U.S. isolates were significantly slower growing, less aggressive, morphologically more unstable, and had a larger range in growth rates than the European isolates (Brasier and others unpublished data; Brasier 2003; Brasier and others, this Proceedings). However, there is no information on fungicide tolerance, spore production or aggressiveness to hosts from the western United States to U.S. isolates. This study examines these phenotypic characters among 12 isolates from a broad range of hosts, locations within California and Oregon, and AFLP genotypes. We examine variation in aggressiveness of isolate populations from California. Additionally, we determined whether measurement of lesions on inoculated coast live oak stems is a good measure of pathogenicity.

Methods

Twelve *P. ramorum* isolates were used in all experiments, unless specified otherwise. These isolates were from a broad range of hosts and locations within California and Oregon, and AFLP genotypes (see Ivors and others 2004). For fungicide tolerance experiments, isolates were grown on vegetable-8 juice agar (V8A) modified with 53 and 2134 $\mu\text{g}/\text{mL}$ phosphite (Agrifos 400; Agrichem, Loganholme, Qld, Australia) at 22 °C. Colony size was calculated as a percentage of the control after 10-days growth on agar. Sporangia were produced in Multiwell 24 well plates (Falcon, Franklin Lakes, NJ) containing 350 μL sterile water. All sporangia were counted within the well. Oospores were produced in two separate experiments by pairing the cultures with an A1 and A2 tester isolate on separate carrot agar plates (Brasier and others 2004). A 10-mm square was removed from each mashed paired culture and examined under the microscope. For pathogenicity experiments, bay laurel leaves were

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inoculated with zoospores (Hüberli and others 2003) and coast live oak seedlings were inoculated with agar discs (Dodd and others 2004). Each inoculation was repeated once. Forty-five isolates from three different counties in California were used to inoculate coast live oak seedlings. Stems from four coast live oak trees were inoculated with three isolates as described in Dodd and others(2004). Small sections (0, 0.5, 1.5, 2 and 3 cm from beyond both sides of the longitudinal lesion) were plated onto P₁₀ARP.

Results and Discussion

There was large and significant ($P < 0.001$) isolate variation in all morphological and pathological phenotypes examined (*table 1*). A significant positive correlation ($r = 0.89$, $P < 0.001$) between lesions on bay laurel and coast live oak was found. Two low and three highly aggressive isolates were identified for both hosts. No other linear correlations were found between different phenotypes measured. However, the two low aggressive isolates produced low concentrations of sporangia, and vice versa for the highly aggressive isolates. All three highly aggressive isolates were able to grow on the highest concentration of phosphite. One highly aggressive isolate was identified as having high fungicide tolerance, and produced abundant sporangia and oospores. It is these types of isolates that need to be used in control studies, host resistance screening and recombination analysis.

Inoculation of coast live oak seedlings with 45 isolates from three populations (Santa Cruz, Sonoma and Monterey Counties) in California revealed that Santa Cruz isolates were more pathogenic than those from the other two counties. In these inoculations, Pr-102 and Pr-52 routinely used in coast live oak (Dodd and others 2004) and bay laurel (Meshriy and others, this Proceedings) inoculations were low to moderately highly aggressive. Hosts identified as being less susceptible with these two isolates need to be screened against a range of highly aggressive isolates to confirm if they are robustly resistant. Population variation in a range of other phenotypes is currently being investigated.

We observed that after removal of the bark from coast live oak stems, recovery beyond macroscopically visible lesions is minimal for *P. ramorum* (*fig. 1*). The recovery of the pathogen from apparently beyond the lesion (2 cm for 2 percent of stems) is explained by some stems producing lesions that are diffuse with non-defined lesion margins. This is in contrast to what has been found for *P. cinnamomi* where the pathogen has been recovered from beyond well-defined lesions of between 3 to 6 cm or more (Hüberli and others 2002a, b).

Table 1—Mean isolate range and P-values for different morphological and pathological phenotypes of 12 isolates of *Phytophthora ramorum* from California and Oregon.

Phenotype	Isolate range	P value
Growth on phosphorus acid (% of control) at:		
53 _g/mL V8A ^a	50-92	< 0.001
2134 _g/mL V8A	0, 10-22	< 0.001
Sporangia production (no./mL)	205-8863	< 0.001
Oospore production	0, + - +++ ^b	NA
Bay laurel lesion area (mm ²)	0, 1-31	< 0.001
Coast live oak lesion length (mm)	19-52	< 0.001

^a V8A, vegetable-8 juice agar

^b Numbers of oospores observed within a 10-mm square of the carrot agar directly beneath the mashed paired isolates; + = few oospores, +++ = many oospores

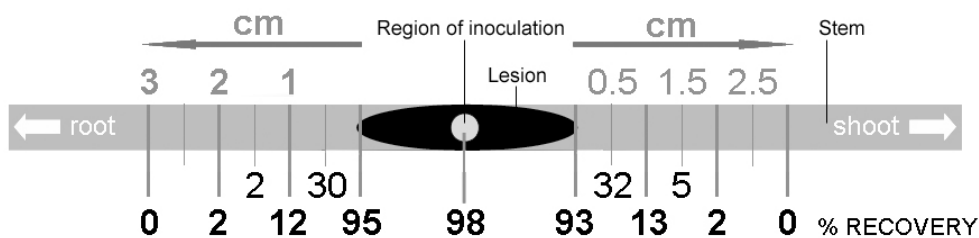


Figure 1—Recovery (percent of 60 stems) of *Phytophthora ramorum* on *Phytophthora*-selective agar medium (P₁₀ARP) from beyond a well-defined, visible lesion of a wound inoculated *Quercus agrifolia* (coast live oak) stem.

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