

Spatial Variation in Effects of Temperature on Phenotypic Characteristics of *Phytophthora ramorum* Isolates From Eastern Sonoma County¹

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Abstract

Quantifying the growth rates of plant pathogens in the laboratory can be useful for predicting rates of disease spread and impact in nature. The purpose of this study was to examine phenotypic variation among isolates of *Phytophthora ramorum* collected from a foliar host plant species, *Umbellularia californica* (California bay laurel), naturally-occurring in localities in three regions (southwest, east, northwest) in a 275 km² study area in Sonoma County. These regions differ topographically, in history of infection by *P. ramorum*, and in microclimate and plant community composition.

We quantified phenotypic variation among *P. ramorum* isolates among regions, plots, and individual trees, allowing us to detect host-plant effects on *P. ramorum* phenotype, and to detect geographic structure in phenotypic characteristics. It also allowed us to test the hypothesis that North American isolates of *Phytophthora ramorum* show relatively high levels of phenotypic variation despite their low levels of genetic variation.

Isolates were collected in spring 2006, after a relatively wet period favorable to *P. ramorum* growth. Growth of 37 different isolates, one or two per tree, from 25 California bay laurel trees from 15 plots was measured on V8 agar plates at four different temperatures (16, 20, 24, 26°C). Each plate was scanned five times over 12 days (day 2, 5, 7, 9, 12). Colony diameter was quantified at each time point using image analysis software (NIH Image). Change in colony diameter over time was analyzed using linear regression of diameter versus day. Growth was linear through day 12 for all replicates, allowing us to estimate growth rate using the slope from each regression. We used growth rate (slope value) and colony size (diameter at day 12), as dependent variables in analyses with region, plot, tree, and isolate as nested grouping factors that were crossed with the grouping factor of treatment temperature in a mixed model analysis of variance.

Growth patterns at 16 to 24°C showed similarities for all isolates, where growth extended horizontally along the agar surface without extending vertically. In some isolates, one or more distinct rings, indicating high densities of chlamydospores, were detected after a few days of growth. Growth at 26°C was substantially different than at the lower temperatures. In many

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cases, the filaments grew upwards away from the agar, and in others, they extended much more thinly away from the origin than observed at lower temperatures. It appeared that this temperature approached the thermal limit for *P. ramorum* under these conditions. For this reason, the 26°C treatment was excluded from further analysis.

We found significant variation in colony size over time among regions, trees within a locality, and among individual isolates. Colonies from the southwest region grew faster than those from the north and east. Growth was significantly greater at 20°C and 24°C than at 16°C. Differences among regions and trees depended on temperature, and individual isolates varied in effects of temperature on colony size.

A multiple regression analysis revealed that *P. ramorum* growth was negatively related to longitude (more rapid growth for isolates from cooler, western plots than from hotter, eastern ones) and elevation, but positively related to Topographic Moisture Index. This suggests that the effect of source environment on growth can persist over a considerable length of time (months in the laboratory).

In conclusion, we found significant variation in *P. ramorum* phenotypic characteristics across three scales within a relatively small geographic area. This high level of phenotypic plasticity may in part explain the invasion success of this pathogen. Accounting for this variation may play an important role in predicting future disease spread.

Key words: *Phytophthora ramorum*, growth rate, California bay laurel.