

Comparative Epidemiology of NA1 and EU1 *Phytophthora ramorum* Isolates from Curry County, OR¹

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Abstract

The 2015 detection of the *Phytophthora ramorum* EU1 lineage in Oregon forests poses a new threat to sudden oak death management in Curry County. EU1 may be more aggressive and spread at a faster rate than has been observed for NA1 over the 17 years it has been managed in Oregon forests. EU1 may also infect some hosts, notably conifers, at a greater frequency. To assess any additional risk posed by EU1, we performed field surveys assessing the distribution and frequency of understory infection surrounding SOD-infested trees. We also conducted laboratory assays testing for epidemiologically relevant differences between Curry County NA1 and EU1 isolates.

To determine if the EU1-infested sites were larger upon detection, or if EU1 was infecting hosts at a greater rate, we established transects 20 m uphill, downhill and perpendicular to a confirmed, SOD-infested tanoak tree presumed to be the primary inoculum source contributing to understory infection at a site (7 sites per lineage). In 5 m² blocks we recorded the presence of understory hosts and collected samples for plating in selective media to confirm infection by *P. ramorum*. Recovery of *P. ramorum* from understory vegetation declined with distance from the primary source of inoculum in both EU1 and NA1 sites. EU1 sites were the same size as NA1 sites upon detection, having similar disease incidence at a given distance from the site center (Wilcoxon rank-sum test; $p = 0.38$). Tanoak was abundant and was the most commonly infected host at both NA1 and EU1 sites. There was no difference between the recovery rates of either lineage for all hosts (Pearson's test for independence at $\alpha = 0.05$). *P. ramorum* was not recovered from conifers.

To complement field studies investigating rates of spread of the NA1 and EU1 lineages, we tested for epidemiologically-relevant differences between Curry County NA1 and EU1 isolates, with the following objectives:

- 1) Assess variation in isolate aggressiveness.

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- 2) Determine sporangial concentrations needed for 50% disease incidence on epidemiologically important hosts and conifers as an indication of greater dispersal capacity or infection rates.
- 3) Determine optimum temperatures for sporulation from rhododendron as an indication of potential for earlier sporulation.

For objective 1, we artificially inoculated tanoak, *Notholithocarpus densiflorus*, stems and rhododendron and bay laurel, *Umbellularia californica*, leaves with mycelial plugs. Leaves and stems were incubated at 20 °C for 7 days before measuring lesion area (leaves) or length (stems). Consistent with prior studies, EU1 isolates were, on average, more aggressive on rhododendron, however some isolates were notably less or more aggressive than others within their lineage. Lesion size on rhododendron was positively correlated with lesion size on bay laurel leaves and tanoak stems.

For objective 2, non-wounded leaves, tanoak sprouts, or conifer seedling-tips (3 per host per concentration) were dipped tip-down into mixed-isolate sporangial suspensions diluted to various concentrations. These were then incubated for 7 weeks prior to plating in selective media to confirm infection. Two sets of assays were performed: one in the spring using Douglas-fir (*Pseudotsuga menziesii*), Japanese larch (*Larix kaempferi*), and rhododendron; and one in the summer using tanoak, bay laurel, and rhododendron. There was no difference between the two lineages in the number of sporangia needed to cause 50% disease incidence on tanoak, bay laurel, or rhododendron. High infection rates of Japanese larch and rhododendron were observed at relatively low concentrations in the spring; greater concentrations are needed for infection on Douglas-fir, though results were too variable to analyze. Preliminary results of a new assay indicate no difference between the two lineages in the number of zoospores needed to produce infection on conifers.

For objective 3, plug-inoculated rhododendron leaves were incubated for 7 days at 20 °C to allow for infection establishment. Leaves were then rinsed and placed in growth chambers set between 4 and 20 °C for one week. Sporangia were rinsed, filtered and counted; the leaves were scanned and lesion area was assessed. Lowest sporulation was observed at 4 and 20° C, with greatest sporulation being observed at 8 °C for some isolates. On average, EU1 isolates sporulated at cooler temperatures, however this was highly dependent upon the isolate used.

Overall, greater lesion sizes and greater capacity to produce sporangia at lower temperatures indicates the EU1 lineage may pose a greater threat to Oregon forests, however some isolates performed more similarly to the opposing lineage.