Comparative Epidemiology of NA1 and EU1 *Phytophthora ramorum* isolates from Curry County, OR

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Introduction

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 over the 17 years NA1 has been managed in Oregon forests[1]. EU1 may also infect some hosts, notably conifers, at a greater frequency.

To compliment 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.
2) Determine sporangial concentrations needed for 50% infection 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.

Methods

Objective 1:
Tanoak stems and rhododendron and bay laurel leaves were wound-inoculated using mycelial plugs. Leaves/stems were incubated at 20°C for 7 days before measuring lesion area (leaves) or length (stems).

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 (three reps each): one in the spring using Douglas-fir, Japanese larch, and rhododendron; and one in the summer using tanoak, bay laurel, and rhododendron.

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.

Variation in Isolate Aggressiveness

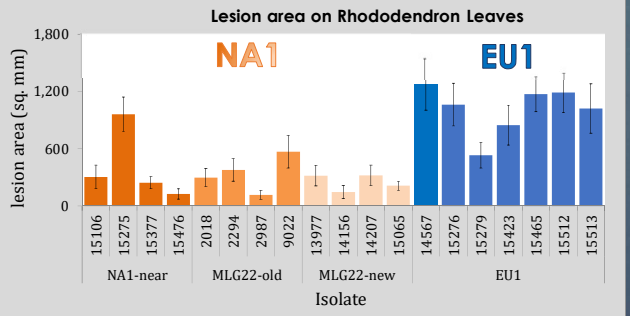

Fig. 1. Lesion size for isolates wound-inoculated onto rhododendron then incubated for 7 days at 20°C. To capture some of the variation within the NA1 lineage, we screened isolates from the following groups: "**NA1-near**" – isolates from within the same drainage as the EU1 infestation; "**MLG22-old**" – isolates determined by Kamvar et al. (2015) to be the most common and persistent multilocus genotype (MLG) in Curry County, recovered between 2001 and 2004; and "**MLG22-new**" – MLG.22 isolates recovered between 2014 and 2016. Bars represent standard error.

Number of Sporangia Required for Infection

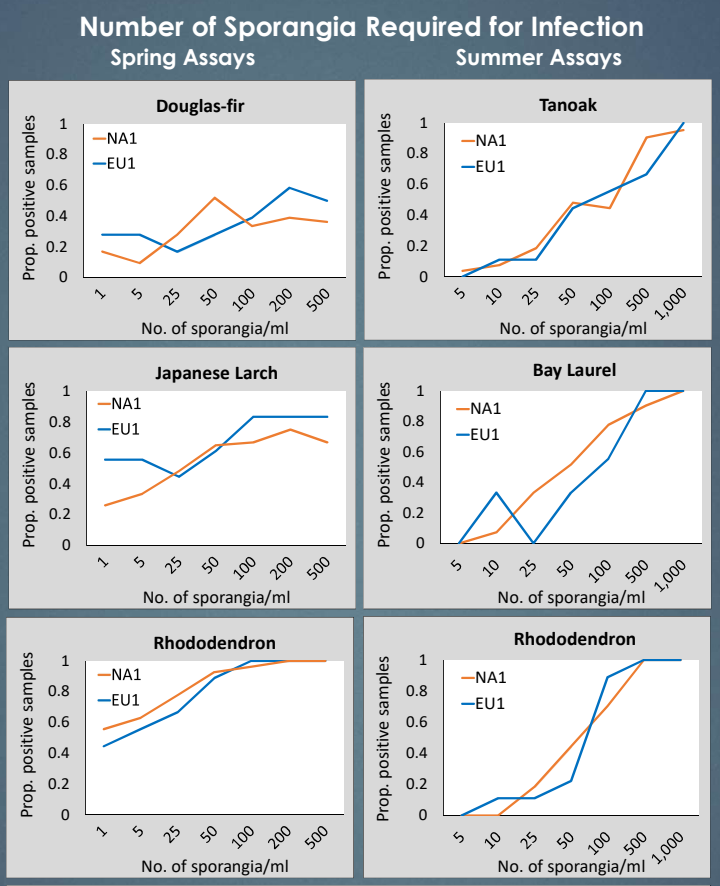

Fig. 2. Disease incidence for leaves, and sprout or seedling tips dipped in mixed-isolate sporangial suspensions. Each isolate group (EU1, NA1-near, MLG22-old, and MLG22-new) was tested separately; no significant difference in incidence was observed between the different NA1 groups and data were combined. Assays for Douglas-fir, Japanese larch, and rhododendron were performed in the spring; assays using tanoak, bay laurel, or rhododendron were performed in the late summer. Each assay was performed three times.

Optimum Sporulation Temperature

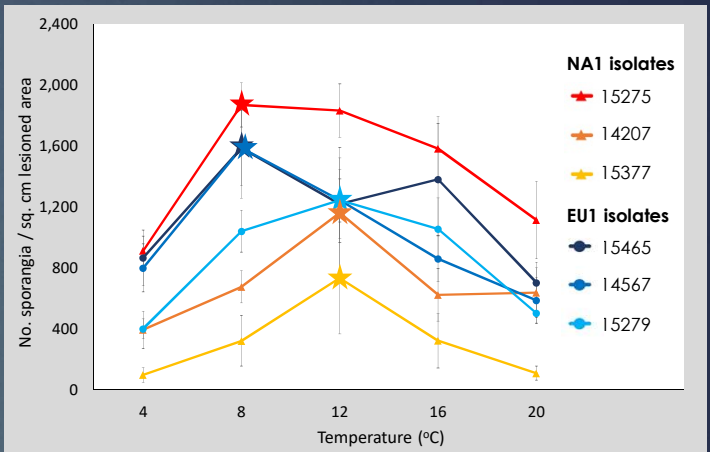

Fig. 3. Number of sporangia produced per square cm lesioned area from week-old inoculated rhododendron leaves incubated at various temperatures for an additional week. Temperature at which maximum sporulation was observed is indicated with a star. Bars represent standard error.

Results & Conclusions

- 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 (Fig. 1). Lesion size on rhododendron was positively correlated with lesion size on bay laurel leaves and tanoak stems (data not shown).
- There was no difference between the two lineages in the number of sporangia needed to cause 50% infection on tanoak, bay laurel, or rhododendron (Fig. 2, summer assays).
- 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 (Fig. 2, spring assays). Preliminary results of a new assay indicate no difference in the number of zoospores needed to produce infection on conifers.
- On average, EU1 isolates sporulated at cooler temperatures, however this was highly dependent upon the isolate used (Fig. 3).
- 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, notably NA1 isolate 15275 and EU1 isolate 15279, performed more similarly to the opposing lineage.

References

1. Manter et al. 2010. Virulence, sporulation, and elicitin production in three clonal lineages of *Phytophthora ramorum*. Physiological and Molecular Plant Pathology 74:317-322.
2. Kamvar, et al. 2015. Spatial and temporal analysis of populations of the sudden oak death pathogen in Oregon forests. Phytopathology 105:982-989.

Acknowledgements

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