

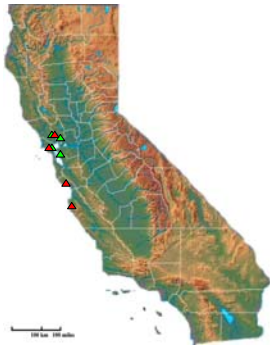
Summer survival of *Phytophthora ramorum* in California bay laurel leaves

Elizabeth J. Fichtner¹, David M. Rizzo¹, Shannon C. Lynch¹, Jennifer Davidson², Gerri Buckles³, and Jennifer Parke³

¹Dept. of Plant Pathology, University of California Davis; ² Dept. of Ecology, Evolution, and Conservation Biology, University of Hawaii; ³ Dept. of Crop and Soil Science, Oregon State University

BACKGROUND

In California, Sudden Oak Death manifests as non-lethal foliar lesions on bay laurel, which support sporulation and survival of *Phytophthora ramorum* in forest ecosystems. Infected bay laurel leaves are more likely to abscise than uninfected leaves, resulting in an accumulation of inoculum at the forest floor. The phenomenon of site-influenced survival of *P. ramorum* within bay laurel has been documented in both a redwood-tanoak forest and a mixed-evergreen forest at Jack London State Park and Fairfield Osborne Preserve, respectively. Summer foliar survival in attached symptomatic leaves is higher in the redwood-tanoak forest than in the mixed-evergreen forest, yet these two sites are located only 5km apart on opposite sides of Sonoma Mountain. The underlying factors mediating site-influenced epidemiological differences are yet unknown. This study focuses on summer pathogen survival associated with bay laurel in redwood-tanoak and mixed-evergreen forests with specific objectives including: i) detection of *P. ramorum* in litter and soils throughout summer, ii) quantification of chlamydospores on attached symptomatic leaves, and in fresh and aged litter, iii) determination of chlamydospore germination, and, iv) assessment of pathogen survival within litter and canopy leaves, addressing the location of viable inoculum within foliar tissues. Trees were tagged for repetitive sampling in redwood-tanoak and mixed-evergreen forests and sampled in May and August 2006.



- ▲ Redwood-tanoak forests
- ▲ Mixed-evergreen forests
- ▲ Jack London State Park
- ▲ Henry Cowell Redwoods State Park
- ▲ Big Sur Pfeiffer State Park
- ▲ Samuel P. Taylor State Park
- ▲ Fairfield Osborne Preserve
- ▲ Skyline Wilderness Park
- ▲ China Camp State Park
- ▲ Pacheco



OBJECTIVES

In both redwood-tanoak and mixed-evergreen forests:

1. Detect inoculum of *P. ramorum* in litter and soils.
2. Quantify chlamydospores on surfaces of:
 - ◆ Attached symptomatic leaves
 - ◆ Fresh leaf litter
 - ◆ Aged leaf litter
3. Determine chlamydospore germination potential.
4. Assess pathogen survival within litter and canopy leaves:
 - ◆ determining recovery with PCR and isolation
 - ◆ addressing propagule types present

FIELD SAMPLING STRATEGIES

Time: May and August 2006; collection at all sites conducted within 48 hours.

Locations: 4 redwood-tanoak forests and 4 mixed-evergreen forests

Tagged Trees: 10 trees per site for repetitive sampling

- Samples:
- ◆ soil and symptomatic bay leaf litter for bulk baiting.
 - ◆ attached symptomatic and asymptomatic (control) leaves.
 - ◆ fresh and aged symptomatic bay litter leaves.

METHODOLOGY

1) Bulk baiting

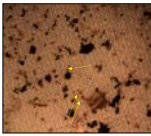
For each tree, a bulked soil sample and a bulked litter sample were baited with rhododendron leaves and soil moisture content was determined for each sample.



Soils and litter were baited with rhododendron leaves. Symptomatic bay leaves were placed on PARPHs.

2) Surface Chlamydospore Determination

One symptomatic attached leaf, one fresh litter leaf, and one aged litter leaf from each of 10 trees/site were individually scrubbed with a moistened toothbrush. The resulting spore suspension was passed through a 35 µm nylon mesh and chlamydospores were counted under a dissecting microscope. Similarly two asymptomatic attached leaves per site were assessed for chlamydospore presence.



Yellow arrows indicate chlamydospores on nylon mesh.

**note: No viable sporangia were detected in spore suspensions in May 2006.

2) Chlamydospore germination

Ten chlamydospores from each site and leaf type (attached, fresh litter, aged litter) were selected at random and individually transferred to PARPH to determine germination.



Chlamydospores were observed 2 weeks for germination.

3) Pathogen recovery

One symptomatic attached leaf, one fresh litter leaf, and one aged litter leaf from each of 10 trees/site were dissected in thirds along the lesion margin, with two of three sections utilized to determine pathogen recovery via PCR and isolation.

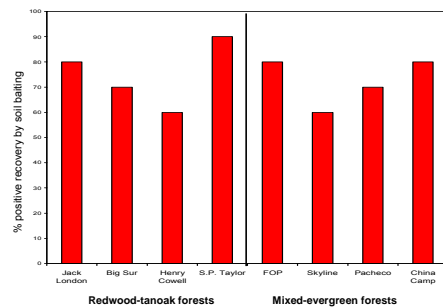


4) Scanning electron microscopy

A section of the lesion border was preserved in FAA (formaline acetic acid), dehydrated, and prepared for SEM.

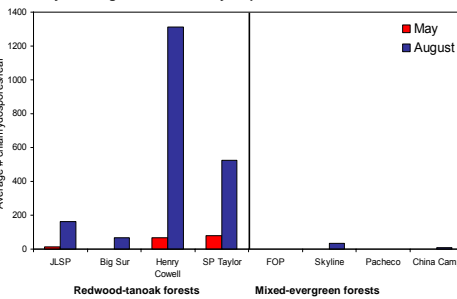
RESULTS

May 2006: Recovery of *P. ramorum* by bulk soil baiting

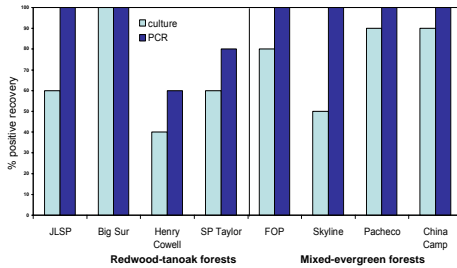


*No recovery of *P. ramorum* from soil in August
 *No recovery of *P. ramorum* from bulk litter baiting in May and August

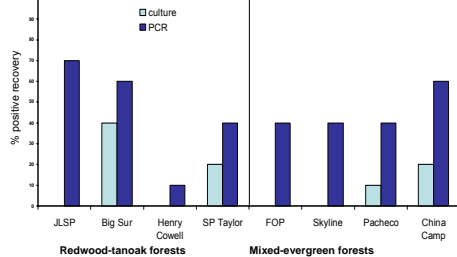
May and August 2006: Chlamydospores on attached leaf surfaces



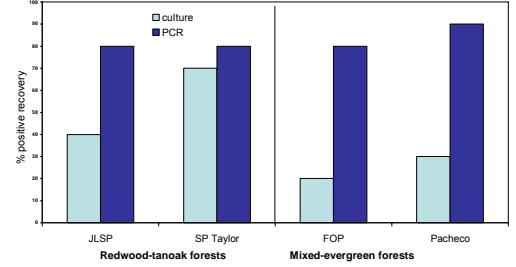
May 2006: Attached symptomatic leaves



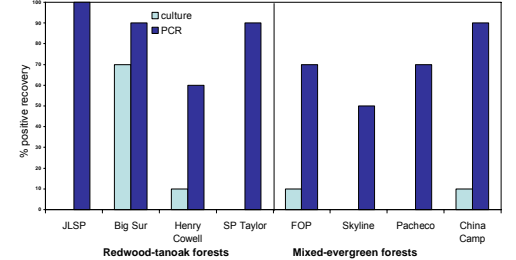
August 2006: Attached symptomatic leaves



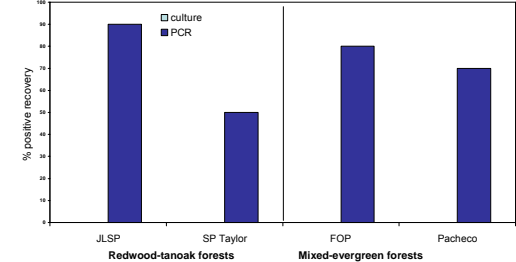
May 2006: Fresh leaf litter (only 4 sites assessed)



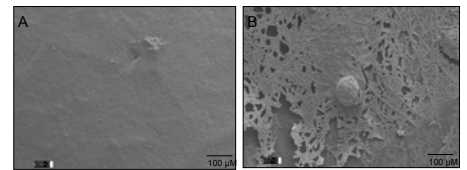
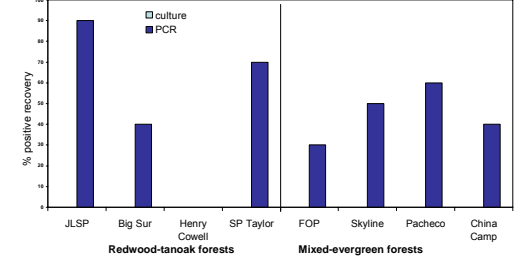
August 2006: Fresh leaf litter



May 2006: Aged leaf litter (only 4 sites assessed)



August 2006: Aged leaf litter



A. SEM photograph of the upper surface of an asymptomatic bay leaf. B. SEM photograph of the upper surface of a symptomatic bay leaf. Note the chlamydospores forming under the waxy cuticle.

CONCLUSIONS

- 1) *P. ramorum* was baited from soils in May but was undetectable by August.
- 2) *P. ramorum* was never baited from symptomatic bay litter, suggesting a lack of viable sporangia in the litter.
- 3) Chlamydospores were more abundant on bay leaf surfaces in redwood-tanoak forests than in mixed-evergreen forests. Chlamydospores form under the waxy cuticle, which may offer some protection from desiccation.
- 4) Recovery of *P. ramorum* from symptomatic attached bay leaves and fresh litter decreased dramatically between May and August. *P. ramorum* was never isolated from aged litter.
- 5) PCR resulted in more positive detections of *P. ramorum* than culture; however, PCR positives may result from non-viable pathogen tissue or residual DNA.

ACKNOWLEDGEMENTS:

Funding: USDA Forest Service-Pacific Southwest Research Station, Gordon and Betty Moore Foundation. Technical Assistance: Andra Westbrook, Akiho Oguchi, Kamyar Aram, Richard Cobb, Kamal Sandhu, Mai Tat, Mitch Corbett, Chris Langsdorf