Metabolite Profiling to Predict Resistance to *Phytophthora ramorum* in Natural Populations of Coast Live Oak

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Coast live oak susceptibility

• Presence of apparently resistant coast live oak (*Quercus agrifolia*; CLO) in stands of high infection and mortality (McPherson et al. 2005).

• Infection rates appear to be slowing (McPherson et al. 2010).

• Exact mechanism of persistence is not known.
Modeling survival and resistance

Trees with cankers < 21 cm have a ≥ 80% probability of survival

≥ 90% probability of survival

Probability of a CLO surviving, i.e. being resistant, based on canker lengths, measured approximately one year after inoculation (2003); actual survival assessed in 2009 (McPherson et al., unpublished).
Chemical mechanisms of resistance in oak

- Putative phenolic biomarkers of resistance have been identified in CLO phloem tissue (Nagle et al. 2011, Ockels et al. 2007).
  - Tyrosol hexoside pentoside
  - Ellagic acid
  - Total phenolics

- Tyrosol inhibits *P. ramorum* growth *in vitro* (Ockels et al. 2007).

- Constitutive (baseline) concentrations of these compounds in the phloem of CLO can be used to discriminate between resistant and susceptible trees (McPherson et al., unpublished).
Phenolic biomarker thresholds (mg g\(^{-1}\) FW) for predicting coast live oak resistance to *P. ramorum*

<table>
<thead>
<tr>
<th>Compound(s)</th>
<th>Threshold at 90% probability</th>
<th>Threshold at 95% probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosol glycoside (tyrosol equivalents)</td>
<td>5.85 ± 1.86</td>
<td>6.72 ± 2.50</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>0.53 ± 0.21</td>
<td>0.62 ± 0.31</td>
</tr>
<tr>
<td>Total phenolics (at 370 nm; ellagic acid equivalents)</td>
<td>6.87 ± 2.73</td>
<td>8.80 ± 3.98</td>
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Threshold levels (± 95% CI) for the three phenolic biomarkers of resistance are given at 90% and 95% probabilities. Levels determined using logistic regression analysis (McPherson et al., unpublished).
Goal and hypotheses

• To identify resistant trees in populations of coast live oak before *P. ramorum* infection.

• Hypotheses
  1. Constitutive levels of phenolic biomarkers will not change seasonally.
  2. Resistant CLO will contain levels of constitutive phenolic biomarkers higher than susceptible CLO.
Testing biomarkers on a naïve CLO population

- Phloem was excised from the main stem of 148, asymptomatic CLO prior to inoculation with *P. ramorum*. Soluble phenolics were extracted from phloem tissues.

- Quantified phenolics were compared to threshold levels for each of the three phenolic biomarkers of resistance.

- Using the survivorship model developed by McPherson et al. we determined the probability of resistance of each tree based on canker lengths.

- To assess the accuracy of these biomarkers at predicting resistance, trees were inoculated with *P. ramorum* and external cankers were measured 10 months after inoculation.

Briones Regional Park, Contra Costa Co., CA (East Bay Regional Park District).
External canker lengths measured 10 months after inoculation. Values are averaged across two inoculation points on each tree. Mean = 62.2 cm; SE = 2.71; N = 148.
Estimated probability of survival

16% of trees have \( \geq 80\% \) probability of survival

Probability of survival based on average external canker lengths measured 10 months after inoculation and model of McPherson et al. (unpublished).
Phenolic biomarkers (mg g\(^{-1}\) FW) do not vary seasonally

<table>
<thead>
<tr>
<th>Compound(s)</th>
<th>Dec 2010</th>
<th>July 2011</th>
<th>Nov 2011</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosol glycoside (tyrosol equivalents)</td>
<td>5.03 (0.599)</td>
<td>4.92 (0.519)</td>
<td>5.02 (0.654)</td>
<td>0.759</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>0.200 (0.018)</td>
<td>0.212 (0.022)</td>
<td>0.397 (0.120)</td>
<td>0.145</td>
</tr>
<tr>
<td>Total phenolics (at 370 nm; ellagic acid equivalents)</td>
<td>4.17 (0.387)</td>
<td>4.21 (0.371)</td>
<td>4.09 (0.552)</td>
<td>0.289</td>
</tr>
</tbody>
</table>

Mean concentration of phenolic biomarkers (SE). P-values determined using repeated measures general linear model (p-value for total phenolics based on log transformed data).
Phenolic biomarkers of resistance

83% of trees with ≥ 80% probability of survival (n = 20) contained one or more biomarkers at the ≥ 90% probability of resistance threshold.
Conclusions

• Based on the survival model of McPherson et al. (unpublished), 16% of CLO in an East Bay naïve area have a ≥ 80% probability of survival.

• Phenology does not significantly affect the concentration of phenolic biomarkers in CLO phloem.

• The presence of phenolic biomarkers of resistance at or above threshold levels in 83% of trees with a high (≥ 80%) probability of survival is promising.

• Such thresholds, as well as the survival model, will probably have to be revised based on the current analysis of naïve trees.

• Identification of resistant trees in natural populations of CLO can be incorporated into disease management plans aimed at preserving resistant trees and may be useful in breeding efforts.
Acknowledgements

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