A Comparative Analysis of Diagnostic Approaches for *Phytophthora ramorum*

Matteo Garbelotto[1], AnnaMaria Vettraino[1], Serenella Sukno[2], Lydia Baker[1], and Amy Smith[1]

[1]University of California, Berkeley. Department of Environmental Science, Policy, and Management, 137 Mulford Hall, Berkeley, CA, 94720-3114; matteo@nature.berkeley.edu

[2]Texas A&M University. Department of Plant Pathology and Microbiology, 2132 TAMU, College Station, TX 77843

Introduction

The timely detection of plant pathogens is a key issue in the fight to stop the undesired spread of microbes associated with the trade of plants and/or plant parts. Timely detection is also an essential component of land surveys if pathogen eradication or preventive protective treatments are in question. While some plant pathogens can be reliably cultured at all times, others can never be cultured. Many microbes, including many plant pathogenic *Phytophthora* species, fall in between the two extremes. As a response to the unreliable growth of some pathogens *in vitro*, researchers, surveyors, and governmental agencies have increasingly turned to molecular diagnostic assays directly from environmental samples in lieu of more traditional sampling techniques. Because of the fast pace at which this field is moving, often techniques are adopted without a significant amount of information on their limitations, drawbacks or benefits. This study was designed to compare the sensitivity and robustness of several diagnostic assays available for the SOD pathogen in a real-world situation.

Experimental design

7 sites were intensively sampled across 3 counties
346 symptomatic plants were collected
37 plant species sampled
165 bay laurel trees were sampled from 5 sites in 3 counties

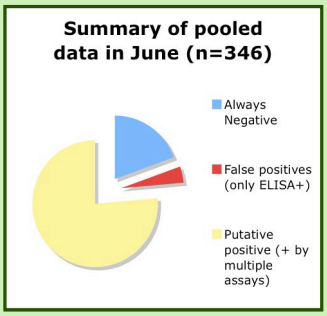
5 Diagnostic methods were tested:
- Plating on CMA-PARP
- Plating on V8-PARP
- CSL Taqman PCR
- Nested PCR
- ELISA (generic *Phytophthora* kit: Agdia, Inc., Elkhardt, IN)

All assays were replicated in two laboratories (UCB and Texas A&M)

All plants were sampled twice, in June and September 2005

Results

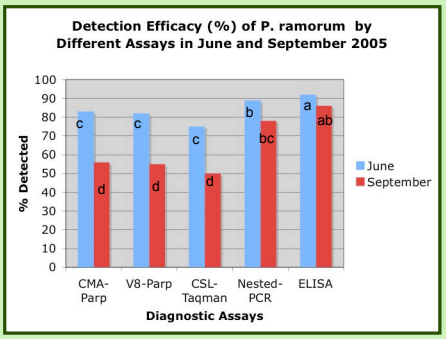
Inferences from pooled data
Fig.1



This UC data was used as baseline for all comparisons

Results, cont'd

Comparing the 5 methods at the two sampling times
Fig.2



Different letters indicate significant differences based on chi-square, P=0.05

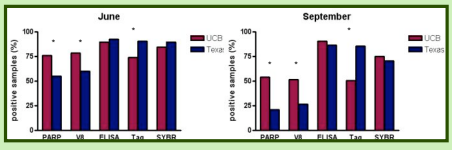
Comparing culturing to molecular methods on a sample per sample basis we found out the following values of overlapping positive results:
Table 1

Correspondance between positives based on cultures (both media combined) and positives obtained by molecular assays.

Comparison	Overlap
Culturing-CSL Taqman	75%
Culturing-Nested PCR	87%
Culturing-ELISA	97%

Comparing results from the two laboratories
Fig.3

Results of Chi-square between UCB and Texas for each method and season.
* indicate significant differences between campuses at P<0.01



Results, cont'd

Comparing results exclusively from bay laurel across five different sites
Table 2

Significance (P values) of Chi-square tests comparing efficacy of detection *of P. ramorum* among five different sites in two months. Values under 0.05 indicate the assay performed at different efficacy levels across the five study sites for that time.

Assay type	P value in June	P value in September
V8-Parp	0.24	0.001
CMA-Parp	0.21	0.005
CSL-Taqman	0.0001	0.0001
Nested-PCR	0.46	0.09
ELISA	0.42	0.41

Conclusions

When sampling based on obvious symptom, a significant number of samples will not be infected by *P. ramorum* (Fig.1)

In general, molecular methods performed better than standard culturing. V8- and CMA-PARP had equal performances. Both culturing methods were strongly affected by season (Fig.2), by lab (Fig.3), and by site sampled if sampling occurred in the Fall (Table 2)

ELISA kits performed extremely well, with an estimated false positive rate of approximately 5% in California coastal forests (Fig. 1). ELISA results were not affected by season (Fig.2), laboratory (Fig.3), or by site (Table 2). They also showed good overlap with culture-positive samples (Table 1)

CSL TaqMan was not any more sensitive than culturing at UCB and was affected by season (Fig.2), it had the lowest overlap with cultured positives (Table 1), and was also affected by the site sampled both in June and September (Table 2). Results were affected by the laboratory performing the test

Nested PCR was as sensitive as ELISA and was not affected by season (Fig.2), laboratory (Fig. 3), or site (Table 2). Its results had a good overlap with cultured positives (Table 1)

Nested PCR had the most desirable features for specific detection of *P. ramorum*. Culturing, the worst (lack of repeatability, strong effect of season, effect of site). The intermediate performance of CSL TaqMan may be due to technical differences between the two labs. ELISA performed very well and has many desirable features (repeatable, not affected by site or season) although it is prone to false positives. Nonetheless, it seems an ideal first screening tool. ELISA negative can definitely be trusted based on this extensive field survey.

This work was supported by the United States Department of Agriculture - Animal and Plant Health Inspection Services.

References

Hayden, K.J., Rizzo, D., Tse, J. & Garbelotto, M. (2004) Detection and Quantification of *Phytophthora ramorum* from California Forests Using a Real-Time Polymerase Chain Reaction Assay. Phytopathology 94, 1075-1083.

Hughes, K.J., Tomlinson, J., Griffin, R., Boonham, N., Inman, A., and Lane, C. (2006) Development of a One-Step Real-Time Polymerase Chain Reaction Assay for Diagnosis of *Phytophthora ramorum*. Phytopathology 96, 975-981.

http://www.aphis.usda.gov/