Development and validation of a rapid molecular detection tool for *Phytophthora tentaculata*

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Introduction

In 2012 the fungal plant pathogen, *Phytophthora tentaculata, known to cause basal rot* was detected in California native plant nurseries.

P. tentaculata currently has a known host range of five plants species, but the number is expanding and extensive host range studies are being conducted (Latham *et al*., personal communication).

Cal State Monterey Bay's Watershed Institute recently detected this pathogen on native plant species at the nursery, Return of the Natives (RON), and within a restoration site. The spread of this pathogen in a natural setting could negatively affect many of California's plant communities.

Many native plant nurseries do not have access to testing methods and obtaining results from outside laboratories can be tedious. Current methods of detection can be cumbersome and inaccurate. However, the recent development of a rapid molecular detection technique, Recombinase Polymerase Amplification (RPA), has been developed for the *Phytophthora* genus (Miles *et al*., 2015)

Objectives:
Utilize four different methods of detection to identify *P. tentaculata* in nurseries to:
1) Determine the amount of time required for each method: qPCR, genus/species specific RPA assays and Agdia Immunostrips.
2) investigate the ease and feasibility of each method
3) correlate detection results of each method across different platforms.

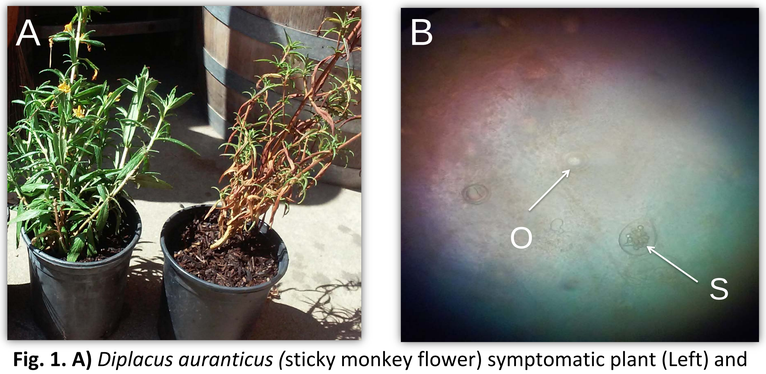

Fig. 1. A) *Diplacus auranticus (*sticky monkey flower) symptomatic plant (Left) and healthy individual (Right).
B) Image of a *P. tentaculata* culture with asexual reproductive structure (sporangia, **S**) and the sexual structures (oospores, **O**).

Acknowledgements

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References

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2. Timothy D. Miles, Frank N. Martin, and Michael D. Coffey. Development of Rapid Isothermal Amplification Assays for detection of *Phytophthora* spp. in Plant Tissue. Phytopathology 2015 105:2, 265-278
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Methods

Samples were collected at a restoration site adjacent to Cal State Monterey Bay property. Symptomatic *Diplacus aurantiacus* root crowns were collected under sterile conditions.

qPCR Probe Detection
-DNA was extracted from the root crown using Plant DNA extraction kit (Qiagen, 3 hours)
-Assembled master mix (5Prime) contained internal *cox* plant specific primers, to confirm successful DNA extraction, and forward primers that bind to *atp9* and reverse primers that bind to *nad9* to identify presence of pathogen (30 Minutes)
-Reactions were ran in the BioRad CFX96 qPCR machine (3 Hours)
Total Processing Time: 6.5 hours

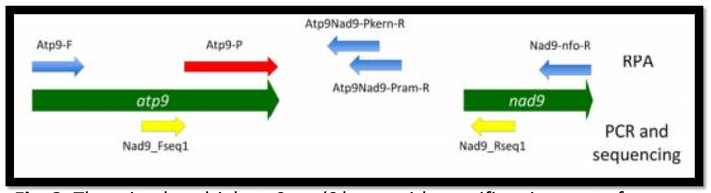

Fig. 2. The mitochondrial *atp9-nad9* locus with specific primer sets for species-specific RPA markers (figure from Miles *et al*., 2015).

Genus and Species Specific RPA
-Using a section of the root crown 5mL of General Extraction Buffer 2 (Agdia Inc., Elkhart, IN) was added
-In sample extraction pouch (Agdia), lyse the sample until a homogenous solution is achieved (10 minutes)
-Assemble a master mix with RPA primers (30 minutes)
-Reaction was ran in the isothermal machine at 39°C (25 minutes)
Total Processing Time: 1.1 hours

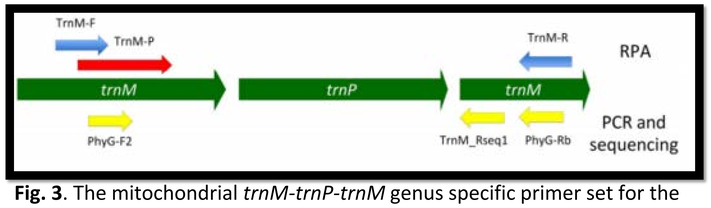

Fig. 3. The mitochondrial *trnM-trnP-trnM* genus specific primer set for the RPA reaction (Miles *et al.,* 2015).

Immunostrip Detection
-5mL of Simple Extraction Buffer was added to root crown section in a sample extraction pouch (Agdia)
-Sample was emulsified until a homogenous mixture was achieved (10 minutes)
-Immunostrip added to emulsified liquid (30 minutes)
-Positive Identification: presence of two lines
Total Processing Time: 40 minutes

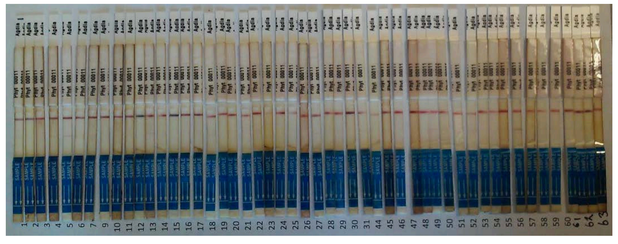

Fig. 4. Immunostrip identification of *Phytophthora* spp. and *Pythium spp.* (56 samples)

Results

qPCR Probe Detection
The qPCR method was accurate and was the method most commonly used even though this was the most tedious process, 6.5 hours.

Genus and Species Specific RPA
The RPA assays were accurate in *Phytophthora* detection, aligning with the qPCR technique. This method was extremely more time efficient with results achieved in 1.1 hours.

Immunostrip Detection
Although results were obtained in a minimal amount of time (40 minutes), immunostrips were inaccurate due to cross reactions with *Pythium spp.* that provided false positives for non-*Phytophthora* samples.

Table 1. Positive detections for *P. tentaculata* using each method

Total	ELISA	qPCR genus	qPCR species	Genus RPA	Species RPA
56	22	15	12	15	6
	40 minutes	6.5 Hours	6.5Hours	1.1 Hours	1.1 Hours

Discussion

After undergoing four separate tests results would indicate the RPA is the best option for rapid detection of a pathogen in a wild setting or in the case of a nursery. The Immunostrips were useful for general pathogen detection they were unspecific. qPCR provides a great amount of information and should be used for validation as well as quantification but is time consuming and requires a considerable upfront investment. Validation using pear baiting and classic culturing technique however proved to be difficult during dry season. These types of assays are necessary for making decisions to save time and money as well as assist with thorough understanding of the pathogen and prevent it from spreading outside of nurseries and restoration sites.

Future Work

The use of additional RPA assays will identify the epidemiology of the pathogen and will in turn be used to map projected spread of disease in correlation to elevation. This will predict the movement patterns of the pathogen and determine if there is a correlation between pathogen presence, specified elevations and areas of tarning.

Fig. 5. Map of the *Lightfighter site* that has the positive samples (red) tested with qPCR and all other samples collected (blue). Maps like these will be used as preliminary data for the correlation to elevation for epidemiological studies.