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From Lab to Field: A Molecular Test for detecting *Phytophthora* spp. in Nursery Water Systems

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Abstract:

Phytophthora pathogens, causal agents of root and crown rots in many ornamental species, are a recurrent challenge in nursery production, generating losses, affecting plant health, and releasing pathogens of regulatory concern into trade and natural ecosystems. Efforts to control pathogen spread are costly, making imperative the development of reliable and fast diagnostic tools. The recombinase polymerase assay (RPA) is a rapid and accurate method for detection of *Phytophthora* spp. that has been previously optimized for plant tissue. In this study, we optimized the RPA assay for *Phytophthora* detection directly from water. We used serial dilutions ranging from 10⁵ to 10 spores/mL from nine different *Phytophthora* species frequently found in nursery systems, and filtered suspensions onto glass microfiber filters. We confirmed the specificity of *Phytophthora* RPA *TrnM* mitochondrial primers and probe on all tested species, and determined 100 spores/mL as the limit of detection of the assay. Current studies evaluating assay detection efficacy under real life scenarios include testing artificially inoculated water at nursery scale collection tanks, and from leachate water of potentially infected plants. Assay robustness at large scale settings will provide improved tools for early *Phytophthora* spp. detection in nursery production, preventing pathogen spread into trade and natural ecosystems.

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