

Development of Reagents for Immunoassay of *Phytophthora ramorum* in Nursery Water Samples¹

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Abstract

Current regulations under the August 6, 2014 USDA APHIS Official Regulatory Protocol (*Confirmed Nursery Protocol: Version 8.2*) for Nurseries Containing Plants Infected with *Phytophthora ramorum* mandates the sampling of water in affected nurseries to demonstrate they are free of *P. ramorum*. Currently, detection of *P. ramorum* in these samples requires baiting periods followed by pathogen growth and/or expensive nucleic acid molecular detection methods. A rapid detection tool would greatly reduce the time, cost, and effort needed to demonstrate that nurseries are free of *P. ramorum*. To meet this need, we are currently developing rapid antibody detection tools for *P. ramorum* in nurseries, focusing on detection in irrigation water, surface water, and irrigation sources.

Recently published information has revealed extracellular *Phytophthora* proteins with hyper-variable domains that represent promising targets for development of species-specific diagnostic immunoreagents. Sequence alignments across numerous *Phytophthora* species have identified *P. ramorum*-specific antigenic domains within these proteins, providing an opportunity to target these domains for generation of antibodies for detection of zoospores, sporangia, and possibly chlamydospores. Using this information, we have made polyclonal antibodies against recombinant peptides that are reactive against *P. ramorum* zoospores and sporangia, respectively, in Western Blot and ELISA assays, as well as live cell immunofluorescence experiments. Sensitivity and specificity testing is in progress against a number of *Phytophthora* spp. that have been reported to be identified in nursery and surface water samples from across the U.S. Peptide antigens that generate the required specificity for *P. ramorum* in polyclonal antibody testing will be used for monoclonal antibody production for sandwich ELISA and immunostrip assays.

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