Development of DNA Aptamers for Field Detection of *Phytophthora ramorum*

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Sudden oak death (SOD) is caused by the oomycete *Phytophthora ramorum*. Many trees and shrubs are hosts to *P. ramorum* but are not killed by the pathogen, and thus remain a source of inoculum for spread of the disease. These hosts, which include trees as well as shrubs found in nurseries, are either asymptomatic or display a wide range of symptoms. This makes isolation of the disease and spread reduction extremely difficult. While diagnostic tests exist to detect *P. ramorum* in samples obtained from the field, none offers a rapid, sensitive method to detect *Phytophthora* directly in the field or nursery setting. The ability to rapidly detect the presence of *P. ramorum* in the field or nursery would greatly enhance the ability to control the spread of the disease.

We are developing a protein-based field detection system for *P. ramorum*. Briefly, our approach is to develop DNA aptamers that will detect *P. ramorum* proteins. DNA aptamers are small, single-stranded DNA molecules that fold into 3-dimensional structures that bind to proteins with high affinity and specificity. DNA-based reagents offer increased stability, affinity and specificity over traditional antibody-based methods, and thus exhibit optimal flexibility for development into a field tool. In addition, identification of proteins instead of the DNA allows for field testing, since secreted or extracellular membrane proteins can be detected directly and do not require the extraction and manipulation of DNA samples.

We have searched the genome of *P. ramorum* that was generated at the DOE Joint Genome Institute to identify genes that encode for proteins that are found at the plant-pathogen interface. The main focus for target selection was a family of highly conserved proteins called elicitins, which are small (roughly 98 amino acid) proteins secreted by all *Phytophthora* species. Elicitins are one of the most abundant proteins found at the plant-pathogen interface. Some elicitins have the ability to spread systemically throughout both susceptible and resistant plants, while others remain at locally at the site of infection. Comparative sequence analyses have been extensively performed on elicitins from over 30 species of *Phytophthora*, and while this family is highly conserved, considerable variation exists, primarily in amino acids on the outside of the protein structure. While elicitins are found in all *Phytophthora* and some *Pythium* species, they have little sequence similarity to any other known proteins. These characteristics make elicitins optimal biomarkers to detect the presence of *Phytophthora*. Additionally, we have identified protein targets including signaling molecules, proteases, and protease inhibitors that are involved in penetration and colonization of host tissue, suppression of host defenses, and induction of defense response and disease symptoms.

We have begun cloning these genes and producing recombinant proteins. These proteins will be used to generate DNA aptamers, which will enable to creation of a rapid field assay for the detection of *P. ramorum*. This work is funded by the USDA Forest Service, Pacific Southwest Research Station (No. 03-1A-11272138-340).