Development of DNA Arrays for Diagnosis and Prediction of Almond Diseases

Project No.: **08-PATH2-Browne/Kluepfel**

Project Leaders: Greg Browne
USDA-ARS
Crops Pathology and Genetics Research Unit (CPGRU)
Department of Plant Pathology
Univeristy of California, Davis
One Shields Avenue
Davis, CA 95616
(530) 754-9351
gregory.browne@ars.usda.gov

Dan Kluepfel
USDA-ARS
Crops Pathology and Genetics Research Unit (CPGRU)
Department of Plant Pathology
Univeristy of California, Davis
One Shields Avenue
Davis, CA 95616
(530) 752-1137
daniel.kluepfel@ars.usda.gov

Project Cooperators: Leigh Schmidt, USDA-ARS, CPGRU, University of California, Davis
Takao Kasuga, USDA-ARS, CPGRU, University of California, Davis
Ali McLean, USDA-ARS, CPGRU, University of California, Davis
Ravi Bhat, Plant Pathology, University of California, Davis

Objectives:

Accurate diagnosis of plant diseases usually requires laboratory work to confirm the presence of suspected pathogens. Traditionally, many plant pathogens have been diagnosed by isolating them in culture (e.g., in Petri dishes) and identifying them based on their appearance. Increasingly, DNA sequencing is performed on DNA of the cultured suspects to confirm their identity and further characterize them. Also, with recent improvements in DNA purification and amplification technology (i.e., with the polymerase chain reaction [PCR]), it is possible to obtain DNA of many organisms directly, without culturing. This DNA can then be sequenced to identify or otherwise characterize the organisms of origin.

Although DNA sequencing is powerful in its ability to identify and characterize organisms, it is expensive. The expense becomes very important when one needs to diagnose a large number of samples or identify a large number of organisms that may be contributing to a disease complex such as replant disease (RD) (i.e., see 2008 Proceedings Report of Browne et al., "*Developing Improved Strategies for Management of Replant Problems*"). DNA array technology, once developed and optimized, can reduce the cost of DNA fingerprinting with little loss in precision. The reduced cost lends itself to high-throughput and repeated application. Our specific objectives are:

1. To develop diagnostic DNA arrays that detect key soilborne almond pests and pathogens.
2. To develop diagnostic DNA arrays that characterize and identify members of soil-borne microbial communities mediating almond replant disease.

Interpretive Summary:

In the first months of this project we have focused on extraction and purification of the genomic DNA and DNA fragments to be arrayed. Our collection of RD-associated bacteria, fungi, and *Pythium* species, as well as *Phytophthora* spp., that cause crown rot, are being used for this. We also are using suppression subtractive hybridization (SSH, a PCR-based technique), as a novel approach to identify DNA fragments that occur uniquely in association with RD-affected and healthy almond trees; such bands are now being sequenced and are candidates for use on arrays being developed for objective 2.