

FY08 Application for Nursery Research Funding
Washington State Department of Agriculture - Nursery License Surcharge
 (Please use one application packet including the Progress Report page for each proposal.
 You must use our form - failure to do so may result in not funding your project.)

Project Title: Development of New Technologies for the Detection of Phytophthora in Ornamental Nurseries in Washington State

Project Leader: Michael David Coffey

Institution (if any): University of California, Riverside

Mailing Address: Department of Plant Pathology, University of California, Riverside, CA 92521

Project Phone Number: (951) 827- 4764 **FAX Number:** (951) 827-4764 **Cellular/Pager Number:** ()

Note: Project leader or his/her designee must be available at above project phone number on March 2, 2007 between the hours of 10:00-12:00 and 1:00-3:00.

Amount Requested for (FY08) July 1, 2007 to June 30, 2008: \$33,000

Start Date: July 1, 2007

Completion Date: June 30, 2010

(Check One) *New Project* _____

Continuing x

If this is a multiple year project, please estimate and list the following information for each future July 1 - June 30 period listed below through project completion:

| Fiscal Years (FY) | July 1, 2008 to June 30, 2009 | July 1, 2009 to June 30, 2010 | July 1, 2010 to June 30, 2011 | July 1, 2011 to June 30, 2012 | July 1, 2012 to June 30, 2013 |
|-------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| \$ Amount Needed | \$33, 000 | \$25,000 | | | |

If you are increasing the above amounts since your last application, please explain why: The increase is due to the request for an item of equipment costing \$8,000 needed to handle 96 well plates. The salary/benefits component is higher due to the hiring of a postdoctoral researcher Dr Masoomah Peiman at 50 percent time.

*Please list all other sources and amounts of funding for this project for the current year only: (Please notify us by February 15 if other funding has been approved and from where.)

| Source | \$ Amount Applied For | Approved | Pending Date of Notification |
|--------|-----------------------|----------|------------------------------|
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Total Amount Needed to Fund Project (Include all sources) \$58,000

If total amount from all sources is not granted, will you be able to complete the project?

Explain: the project is estimated to take 3 years and requires a postdoctoral researcher at 50 percent time for 2 more years.

Please summarize the purpose of this research: (you may attach additional sheets if necessary or submit this summary in your own format)

Phytophthora species have been a major scourge of the ornamental nursery for decades. In recent years the availability of materials such as Subdue and Aliette have allowed commercial production of high quality plants despite the hidden presence and incipient spread of this most destructive of plant pathogens. Unfortunately these highly effective fungicides do not eliminate the problem but rather effectively suppress the processes of growth and sporulation which ultimately lead to development of disease symptoms. As a consequence old enemies such as *Phytophthora cinnamomi*, *P. nicotianae*, *P. cactorum*, *P. citrophthora*, *P. citricola*, *P. cryptogea* and *P. drechsleri* have continued to invade, spread and hibernate in the nursery environment as resting spores (chlamydospores, oospores) and even as incipient infections. With the added problem of the emergence and spread of new species such as *P. ramorum* and *P. kernoviae*, subject to stringent quarantine restrictions, and with a similar potential to spread throughout the industry, it becomes imperative to develop clean stock programs. However, it is critically important that all plants entering a clean nursery operation are free of the pathogen. Without this assurance newly introduced *Phytophthora* pathogens will multiply and spread within the operation. Unfortunately traditional diagnostic methods are not sufficiently sensitive or reliable to detect *Phytophthora* especially if fungicides have been used. In particular, all *Phytophthora* species causing problems on ornamentals are present in soil or potting mixes. Current detection methods focus primarily on above ground detection on foliage and stems. However, emerging technologies have the potential to detect even a few resting spores present in soils or potting mixes. The research is needed to bring together the component technologies and evaluate and compare alternative methods. The strategies to be evaluated are 1) the use of new and improved species-specific antibodies in a lateral flow device and 2) DNA extraction with kits designed for potting mixes or soils followed by use of species-specific primers to detect and differentiate different species. Two detection systems will be evaluated 1) high density agarose gels to detect RFLP patterns and 2) macroarrays set up on nylon membranes and designed to detect all the the important *Phytophthora* species. Through such technological intervention and detection being available at the gates of the nursery, growers will have increased confidence that their clean stock programs will not succumb due to the unnecessary introduction of *Phytophthora*-contaminated stock.

This research will examine new technologies to develop effective protocols for sensitive detection of *Phytophthora* in soil and potting mixes in which ornamental plants are growing. It is likely that several new products will emerge from the research. The aims are to assist in the development of commercial kits that will be effective against potential *Phytophthora* invasion by allowing early detection and thus avoidance of its introduction. In the absence of such detection methods the clean stock program is unlikely to be successful. With current and future quarantine restrictions likely to be enforced for many years to come, especially with aggressive species of *Phytophthora*, the development, evaluation and commercialization off new diagnostics suitable logistically and sufficiently economic are vital for ornamental nurseries to avoid huge losses and even bankruptcy. The critical need is for *Phytophthora*-specific diagnostic methods that are both accurate, sensitive and affordable.

PROPOSAL

There are several described molecular detection and identification techniques that are currently used in the diagnosis of *Phytophthora* including *Phytophthora ramorum*. All require sophisticated and expensive equipment and highly trained technical staff in order to run them. Some, such as the nested PCR method have proved unreliable. Others based on Real Time (RT) PCR are very sensitive and with great care and proper controls can be effective for detection of *Phytophthora ramorum*. However, they are designed to be used by highly trained scientists in government and university labs dedicated to such diagnosis and are not suitable for routine inspection or commercial application. In any case, the costs of running such operations are very high and unsuitable for commercial nursery operations. For routine use, ELISA-based kits are sold commercially (produced by Agdia, Neogen, Pocket, etc) but they are not even specific for *Phytophthora*, let alone *Phytophthora ramorum* as they can also react with *Pythium* and fungi including *Rhizoctonia*, both of which are commonly found in environmental samples from nurseries. As a result commercial nurseries operate without suitable diagnostic methods in place to allow detection and intervention of *Phytophthora*. Reliance on existing

diagnostics. At our disposal, however, is a large database of different such regions, particularly the ribosomal gene (ITS), *Beta-tubulin* (TUB), elongation factor-*alpha* (ELO) and cytochrome oxidase (COX). These regions have been characterized in the last year as part of a USDA crop biosecurity project with Penn State University. Using these FOUR regions we plan to develop a multiplex DNA-based MACROARRAY technology that will permit parallel identification of different species. We are collaborating with a company, Agdia, in the development of macroarray technology that will eventually form the basis of a diagnostic kit to detect *Phytophthora* species.

The DNA primers and sequences have already been characterized for over 70 species. The next stage involves further screening against different *Phytophthora* species (including *Phytophthora cinnamomi*, *P. cactorum*, *P. citricola*, *P. citrophthora*, *P. cryptogea*, *P. drechsleri*, *P. nicotianae*, *P. kernoviae*, *P. ramorum*). Consequently, the key part of the technological development has been accomplished already. Unlike other existing DNA based methods which have utilized primarily ITS sequences, our macroarray assemblages will incorporate diagnostic sequences of as many as FOUR distinct diagnostic probes variously based on genomic or mitochondrial DNA sequences (ITS, TUB, ELO, COX).

We will use high density agarose gels and specific restriction enzymes to develop unique RFLP patterns from PCR amplicons (ITS, TUB, ELO, COX) for the different species. In its own right this will become a diagnostic method that could be used by a small progressive commercial lab. This species-specific database will also provide the technical information required for high quality definition of a multiplex diagnostic technology such as use of DNA-based macroarrays.

A key part of DNA-based technologies are this type is their sensitivity. Coupled with PCR they potentially can detect the DNA from single spores. Currently, most diagnostic methods have focused on symptomatic plants. However, *Phytophthora* is a soilborne disease. Spores, oospores or chlamydospores, are resident in soil or potting mixes and associated with plant roots. An important key to good diagnostics is isolated *Phytophthora* DNA from soil or potting mixes. An important part of our research will be to develop good DNA extraction protocols for soils and potting mixes. This is highly feasible and TWO soil DNA commercial kits currently are available on the market. We will evaluate these kits, modify them as necessary and evolve good protocols for DNA extraction in horticultural nursery environments.

Expenditure Breakdown:

| Period for July 2007 - June 2008 | |
|--|----------|
| 1. Salaries | |
| M. Peiman, Postdoctoral Scholar (50%) | \$16,836 |
| 2. Benefits (32%) | \$5,388 |
| 3. Equipment (centrifuge for 96 well plates) | \$8,000 |
| 4. Supplies | \$1,776 |
| 5. Travel | \$1,000 |
| 6. Total | \$33,000 |

The information requested on this page will have a direct bearing on whether your research request is approved or denied. Letters of support by the industry are also encouraged.

Note: Funding is not available for general overhead cost.