Enclosure 3

Special Technology Development Program
Progress Report

PROJECT NUMBER: R5-2001-01

PROJECT TITLE: Diagnostic and survey methodologies for *Phytophthora* species associated with oak and related species in forest and woodland plant associations in the western United States.

PROJECT STATUS: Continuing (funds are being requested for the next fiscal year to continue the project)

EXPECTED PROJECT DURATION: Two Years

ORIGINAL EXPECTED COMPLETION DATE OF THE PROJECT: FY 2002 (September 2002)

EXPECTED COMPLETION DATE OF THE PROJECT: FY 2002 (September 2002)

SUBJECT: Invasive Species; Monitoring; Organism Biology; Risk and Hazard

STATUS OF SUBJECT SPECIES: non-native

PROJECT OBJECTIVES:

1. Optimize bark isolation methods for SOD (sudden oak death) *Phytophthora* and possible associated species.
2. Evaluate existing molecular diagnostic tools, including commercial ELISA kits and the PCR-ITS probe already developed for *Phytophthora lateralis*; and develop new, specific molecular diagnostic tools.
3. Evaluate soil, root, and stem baiting methodologies for detection of SOD *Phytophthora* and possible associated species. Sample the air-spora using species-specific molecular probes or primers.
4. Evaluate soil and bark sampling strategies, including time of year, size and number of samples, sampling design, and handling considerations.

BRIEF DESCRIPTION OF THE PROJECT: Describe primary activities for each year, summarizing key accomplishments from prior year(s), this year's activities, and objectives for future years.

Primary activities and key accomplishments for the 1st year include:

1) Obtained isolates of *P. ramorum* (150 approximately) from a variety of plant hosts, plant parts (leaves vs. stems), and ecosystems (different forest types and geographic locations).

2) Developed and thoroughly tested a *ramorum*-specific nested PCR protocol for identification of the pathogen in culture, and in plant tissue. This test is accepted as the official standard for confirmation in California. Developed DNA database necessary for the design of DNA probes. Produced DNA sequences of the ribosomal DNA region ITS for 15 isolates from different hosts and regions. DNA probes have been designed in the form of PCR primers. The novel nested approach using two sets of *P. ramorum*-specific primers enhances sensitivity while reducing the risk of false positives. The primers have allowed for the discovery of many new host species, and the successful detection of *P. ramorum* from rain, stream water and soil.

3) Worked out a standard protocol for isolation of *P. ramorum* from different hosts and substrates, emphasizing prompt culturing, preferably in the field, and controlled incubation temperatures. *P. ramorum* and a "*P. illicis*"-like species are reliably recovered from cankers. Isolation from foliage is less certain. Initial screening with the Garbelotto PCR primers allows isolation effort to be concentrated on samples that are PCR-positive.

4) Developed baseline data on success and reliability of standard isolation techniques, and began a database on the effects of season and other environmental parameters.

5) Started a novel approach to isolate *P. ramorum* from canker exudates. Exudates are plated and DNA analyzed in Rizzo-Garbelotto labs in parallel and results are compared.

6) Made a direct comparison of PCR and direct isolation. The two methods give consistent results, but PCR gives more positives.

7) A direct, side-by-side comparison of direct isolation, OSU PCR-ITS-probe, and commercial genus-specific ELISA kit is underway. Tissues of tanoak, rhododendron, *Vaccinium*, and assorted other plants have been analyzed.

8) *P. ramorum* has been recovered from soil and streams using several baiting and filtration methodologies. The pathogen is recovered from rain water by filtration and by centrifugation, followed by plating or PCR.

9) Compared several foliage baits for recovery of *P. ramorum* from naturally infested soil. Baits tested included Port-Orford-cedar, red oak, white oak, tanoak, and rhododendron. Some positive recovery was obtained with all species, but Port-Orford-cedar and rhododendron are most reliable.

10) Recovery success from bark was compared by season on the year, with cool seasons giving greatest success. Bark isolation from bark pieces transported to the lab was compared with isolation directly in the field. Both work, but isolation in the field is more reliable, and usually easier.

The objectives for the 2nd and final year (FY 2002) of the project are to:

1) Enlarge the collection and expand the testing.

2) Develop the quantification method.

3) Develop quality control protocols to be applied by the industry.

4) Develop efficient environmental sampling techniques.

CHANGES TO ORIGINAL PROJECT SCOPE OR OBJECTIVES (changes that need to be made to the original proposal and reasons for the changes): **NONE**

ADDITIONS TO ORIGINAL PROJECT SCOPE OR OBJECTIVES (describe additional accomplishments expected from the project)**: NONE**

FHP LEAD CONTACT (FHP person submitting proposal)**:**

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FHP LEAD INVOLVEMENT

	Role	**Time Commitment**
	Coordinate contracting, reporting	

PRINCIPAL INVESTIGATOR(S):

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PRINCIPAL INVESTIGATOR(S) INVOLVEMENT (add lines as necessary)**:**

Name	Role	Time Commitment
Dr. Everett Hansen	co-principal investigator Development and comparison of isolation techniques	30%
Dr. Dave Rizzo	co-principal investigator Develop culture collection; develop isolation techniques; provide baseline data reliability (seasonality success rate)	30%
Dr. Matteo Garbelotto	co-principal investigator Develop molecular diagnostic markers; develop standard sampling and DNA extraction procedure; test sensitivity and reliability of technique and applications	30%

COOPERATORS: None

PRODUCTS AND DUE DATES (from original application form)**:** As sampling designs and methodologies are developed, data will be shared (written progress reports, seminars, workshops, websites) with State and Federal agencies (CDFA, CDF, APHIS, ARS, USDA Forest Service) involved in regulatory aspects of forest health.

STATUS OF PRODUCTS/PRESENTATIONS: All products anticipated for the 1st year of the project have been completed. Current progress on the project has been outstanding.

ACCOMPLISHMENTS TO DATE:

Products: In addition to the development of the *ramorum*-specific DNA primers, products produced during the first year of the project include the publications and technology transfer activities listed below.

Publications:

1) Davidson, J.M., D.M. Rizzo, and M. Garbelotto. 2001. Transmission of *Phytophthora* associated with sudden Oak Death in California. Phytopathology 91: S108.
2) Garbelotto, M., and D.M. Rizzo. 2001. Preliminary studies on chemical and cultural control of Phytophthora associated with sudden oak death. Phytopathology 91: S30.
3) Garbelotto, M., D.M. Rizzo, and J.M. Davidson. 2001. Studies in the genetics of a new Phytophthora species associated with Sudden Oak Death in California. Phytopathology 91: S110.
4) Garbelotto, M., Svhira, P., and D. Rizzo. 2001. Sudden oak death syndrome in California. Perspective on potential control of a new disease. California Agriculture 55: 1.
5) Rizzo, D. M., M. Garbelotto, J.M. Davidson, G.W. Slaughter, and S.T. Koike. 2001. A new Phytophthora canker disease as the probable cause of Sudden Oak Death in California. Phytopathology 91: S76.

Technology Transfer:

1) Protocols developed have been made available upon request to scientists in three countries of the European Union and to the California Department of Food and Agriculture.

2) Numerous training and informational sessions have been organized for professionals, agricultural commissioners and the general public. Examples include:

   a. Met with interested parties to share results and standardize procedures.

   b. Trained federal and state pathologists in handling of samples and isolation techniques.

   c. Presented current SOD information through oral presentations at the annual meeting of the American Phytopathological Society.

   d. Met with Oregon pathologists to design field monitoring protocols.

   e. Presented SOD overview to SAF meeting in Coos Bay OR.

   f. Presented SOD overview to Oregon nursery growers.

FIRST FISCAL YEAR FUNDED: FY 2001

FUNDS OBLIGATED FROM BEGINNING OF PROJECT THROUGH CURRENT FISCAL YEAR: (include both monetary and in-kind, excluding FHP base funding and salaries)**:**

	Item	Requested Funding	Received Funding	Expended Funding
PREVIOUS YEAR (FY 2001)				
Administration	Salary	$54,000		
	Overhead	$0		
	Travel	$3,000		
Procurements	Contracting	$0		
	Equipment	$0		
	Supplies	$9,000		
YEAR TOTALS		$66,000		$66,000

CURRENT YEAR (FY 2002)				
Administration	Salary	$54,000	$54,000	
	Overhead	$0	waived	
	Travel	$3,000	$3,000	
Procurements	Contracting	$0		
	Equipment	$0		
	Supplies	$9,000	$9,000	
YEAR TOTALS		$66,000	$66,000	

FUNDS NOT USED FROM PREVIOUS FISCAL YEAR (If there are unused funds, what is the reason for not using them? How will the project continue without these funds?): NONE

Fiscal Year	STDP Funding Allocated	Funds Obligated	Funds Unused
FY 2001	$66,000	$66,000	$0

EXPECTED BUDGET FOR NEXT FISCAL YEAR (FY 2003): (include both monetary and in-kind, excluding FHP base funding and salaries)**: NONE** (requested FY 2002 dollars would complete the 2 year project)

DIFFERENCE BETWEEN ORIGINAL AND AMENDED REQUESTS AND JUSTIFICATION (the difference between originally requested funds and funds needed based on changes in the budget or scope of the project)**: NONE**

STDP FUNDING NEEDED:

Total estimated additional future funding needed beyond the current (FY 2002) fiscal year: $0
(Requesting $66,000 for FY 2002)

Estimated STDP funding needed in remaining year(s) of the project by fiscal year. Show separately the funding to be requested/provided from other sources.

Fiscal Year	STDP Funding	Other-Source Funding	Source
FY 2002	$66,000	$60,000	OSU, UCD, UCB

$22,000 to each of the three principal investigators