Enclosure 2

Special Technology Development Program
New Project Proposal

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: New

EXPECTED PROJECT DURATION: Two years

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

SUBJECT: Invasive Species_1_ Monitoring _2_ Organism Biology _2_ Risk and Hazard _2_

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 stream 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 PROJECT:

Year 1:

- Develop and compare isolation methods for Sudden Oak Death (SOD) *Phytophthora* based on cultural, ELISA, and molecular techniques.
- Initiate temporal studies into SOD *Phytophthora* activity to optimize isolation techniques.
- Initiate development of procedures for large scale field sampling for multiple species of *Phytophthora*.

Year 2:

- Conduct field surveys in hardwood forests to test techniques.
- Continue temporal tests of sampling techniques for SOD *Phytophthora*.
- Identify *Phytophthora* species found in native hardwood forests.
- Technology transfer to user groups.

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COOPERATORS: None

JUSTIFICATION: Sudden Oak Death (SOD) has recently developed as a serious threat to tanoak (*Lithocarpus densiflorus*), coast live oak (*Quercus agrifolia*), and California black oak (*Quercus kelloggii*). The disease appears to be caused by a previously unknown species of *Phytophthora*. This new pathogen has currently been detected in coastal northern California oak forests from Big Sur to northern Sonoma County. The range of the susceptible host species, however, extends northward to southwest Oregon, southward to Baja California and eastward into the Sierra Nevada. In addition, related oak species such as canyon live oak (*Q. chrysolepsis*) and interior live oak (*Q. wislizenii*) may also be susceptible. The rapid spread of the disease and the aggressive nature of the pathogen suggest that this may be an invasive species. Oak woodlands cover approximately 8 million acres in California (Pavlik et al. 1991) and extend northward through Oregon and Washington. If the SOD *Phytophthora* is an exotic, this new disease has the potential to cause long-term landscape level changes in western oak forests equal to the effects of urban and agricultural expansion. The SOD *Phytophthora* is also associated with mortality of oaks planted as landscape trees. These landscape oaks, many of which are of exceptional size and character, become hazard trees and fuel as they die, necessitating their removal to prevent hazard to humans and property, and to reduce the risk of fire.

Very limited soil sampling in Oregon (Hansen, unpublished data) indicates that there are *Phytophthora* species resident in riparian areas and oak woodlands. In addition, *P. cinnamomi* has recently been isolated from dead chinkapin in California (Rizzo, unpublished), the first report of this omnivorous pathogen from forests in the Western United States. In irrigated situations, additional *Phytophthora* species (e.g., *P. citricola*, *P. cactorum*) cause similar symptoms on various oak species in California (Mircetich et al. 1977). Multiple species of *Phytophthora* are also associated with disease in orchard tree crops in California, Oregon and Washington (Matheron and Mircetich 1985, Ogawa and English 1991). Many of these *Phytophthora* species are probably non-native, having been brought in on planting stock at various times over the past 200 years. The presence and impact of these *Phytophthora* species in native western hardwood forests has never been examined.

The soil phase of the SOD *Phytophthora* has not yet been found, and limited survey results to date in Oregon and California indicate that other, unrelated pathogens can cause similar symptoms on both tanoak and oaks. In this proposal we aim to test and adapt detection and diagnostic technologies developed for other ecosystems to use in the larger effort to protect oak forests and woodlands from the Sudden Oak Death *Phytophthora*, and perhaps associated *Phytophthora* species, and to extend that information to the field survey and monitoring procedures that are critical to the program.

URGENCY: Urgency is very high. The SOD *Phytophthora* is a recently discovered, potentially exotic, pathogen that must be accurately tracked to determine its current range and any expansion of this range. Because of the potential of spread to other areas of the United States with significant oak resources, development of technology to detect the pathogen is critical. Accurate and rapid detection is necessary to develop any control techniques (prevention of movement of infested soil, for example) and monitor their success.

NATIONAL FHP TECHNOLOGY DEVELOPMENT PRIORITY: Priority 4

TECHNICAL COMMITTEE DEVELOPMENT PRIORITY: Priority 1

SCOPE OF APPLICATION: Development of the technology described here will be applicable to detection of *Phytophthora* species in hardwood forests and urban areas of California, Oregon, and Washington. While the proposal directly targets the SOD *Phytophthora*, these methodologies could be applied to other *Phytophthora* species and other geographic areas with modification. The availability of the new methodologies will be crucial for monitoring efforts in other states with a oak resource that are currently outside the known range of the pathogen.

RESEARCH BASIS: The appearance of Sudden Oak Death coincides with heightened concern about *Phytophthora* in forests worldwide (Hansen and Sutton 2000). Interest in *Phytophthora* species in European hardwood forests has been stimulated recently by a new disease of alder (Gibbs 1995; Gibbs et al. 1999), recognition of *P. cinnamomi* as the cause of cork oak mortality in Mediterranean countries (Brasier et al. 1992), and new insights into an old European disease, oak decline (Jung et al. 1996; Jung et al. 2000). One consequence has been systematic sampling of European oak forest soils for *Phytophthora* species. In the process, it has become evident that a number of species are widespread in European forests. These include the ubiquitous *P. gonapodyides* and the recently described *P. quercina,* as well as *P. cactorum, P. cambivora, P. citricola, P. megasperma* and *P. syringae* (Jung et al. 1996, 1999, 2000; Hansen and Delatour 1999). A number of isolates not readily assigned to existing taxa have also been collected in the course of these studies. Three new species that were isolated with sufficient frequency to allow some understanding of population variation and ecology are described in a manuscript in preparation (Jung, Hansen, and others 2000). The isolation and molecular methods discussed below will be informed by and adapted from these previous studies.

METHODS:

Objective 1: SOD *Phytophthora* can be isolated onto selective media from fresh lesions on still living trees. Limited experience to date suggests, however, that success is dependent careful handling of samples and declines rapidly after tree death. Clear guidelines must be established for survey personnel. A sampling protocol will be developed to determine the limits to successful isolation. Samples will be taken from cankers on living, symptomatic trees and dead trees. Wood chips will be taken from trees that have been dead for various lengths of time to determine if *Phytophthora* can survive following invasion of trees by bark beetles, *Hypoxylon* and other organisms. Sampling will be done at least 4 times during the year to determine if there are temporal differences in *Phytophthora* activity. Currently we expect to sample during late summer, early rainy season, late rainy season and mid-summer. This sampling design will be modified as we learn more about pathogen biology.

Objective 2: It may be possible to speed the diagnostic process from bark lesions with molecular diagnostic tools. Commercial antibody (ELISA) kits are available that give a fast indication of the presence of *Phytophthora* species. The kits are not species specific, but could be useful for a quick field diagnosis under carefully defined conditions. We will test the ELISA kits to determine their reliability under SOD *Phytophthora* conditions. Experiments will be conducted to compare results obtained by ELISA with those obtained from direct isolation and PCR-based methods (see below).

A polymerase chain reaction-based protocol for detection of *Phytophthora lateralis* in plant tissues and water has been developed (Winton and Hansen 2000). The procedure reliably detects *P. lateralis* in cedar stem tissues and in roots. Positive reactions were obtained with as few as 200 *P. lateralis* zoospores in water. Similar assays have been developed for some of the European oak *Phytophthora* species (Schubert et al. 1999). We will test the existing *P. lateralis* assay for activity against SOD *Phytophthora*. Because of the reported DNA similarity between the two species, it is possible that the one assay will work for both organisms. We will also develop and test a SOD *Phytophthora* species-specific assay. Base pair deletions in both of the ribosomal DNA ITS regions in *P. lateralis* were used to design complementary PCR primer sequences that amplify a 738 base-pair fragment only if *P. lateralis* DNA is present in the sample. Universal control primers based on conserved sequences of the nuclear ribosomal small subunit are included in a multiplexed reaction as an internal check on the procedure. The universal primers amplify an approximately 550 base-pair fragment that is common to plants, protists, and true fungi (Winton and Hansen 2000).

A new approach recently developed at UC Berkeley for the molecular analysis of wood decay fungi (Garbelotto, personal communication) allows successful DNA amplification from wood. Similar methods will be used to develop a new assay for the SOD pathogen. To date, we have assembled a culture collection of over 30 isolates of this new species that will be useful in screening for variation in the pathogen and development of molecular assays.

Molecular assays that measure the amount of fungus tissue present are often more sensitive than other assays. Approaches include TaqMan real-time PCR, which has become a mainstay of Swiss needle cast monitoring programs in Oregon (Winton et al. 2000), and single copy microsatellite arrays that can be detected and measured with PCR (Groppe and Boller 1997). We will evaluate the utility of these tools for diagnosis and survey of SOD.

Objective 3: Neither direct isolation nor molecular diagnostic tools are reliably effective with soil, root, and water samples. Numerous and fast growing competing microorganisms, and DNA-inhibitory compounds interfere with these direct assays. Instead, a variety of indirect baiting assays have been developed to detect and identify *Phytophthora* species below ground and in water (Erwin and Ribeiro 1996). In the European oak work, isolates were typically recovered from soil samples collected near the bases of oaks. One-liter soil samples were removed to the laboratory, flooded, and baited with very young leaflets of *Q. robur* (Jung et al. 1996, Jung 1998) or twigs of *Chamaecyparis lawsoniana* (Hansen and Delatour 1999). After 2 to 7 days, baits were transferred to selective PARPNH – agar (Jung et al. 1999) or CARP – agar (Hansen and Delatour 1999). Colonies growing from the baits were transferred to V8-agar (V8A) or corn meal agar (CMA) for initial confirmation as *Phytophthora* species. We will test these and similar baits for utility with SOD *Phytophthora* and associated species. Initial tests will use artificially inoculated soils, and successful procedures will be tested in forests and streams. Sampling will be done at least 4 times during the year to determine if there are temporal differences in *Phytophthora* activity.

Objective 4: Information gathered from objectives 1-3 will be used to develop sampling strategies at landscape scales. We will develop protocols that include spatial distribution of samples and sample sites, size and number of samples, time of year, and sample handling considerations. These sampling protocols will be evaluated in a range of oak woodlands in California and Oregon. These woodlands will include symptomatic and non-symptomatic forests in areas within and beyond the known range of the SOD *Phytophthora*. In woodlands with symptomatic trees, samples will be taken from wood, soil and streams. In areas without SOD, sampling will be restricted to soil and water. Additional data collected along with *Phytophthora* samples will include forest type, slope, aspect and individual tree data (species, crown class, crown condition, diameter). All *Phytophthora* species recovered during these surveys will be identified to the species level using culture morphology and molecular methods (e.g., sequences of ITS, Cooke et al. 2000).

MEASURES OF SUCCESS:

Standard of Success:

1) Publication of techniques developed in refereed journals.
2) Use of techniques by field personnel to accurately and rapidly determine the range of *Phytophthora* species in oak woodlands.
3) Use of techniques to monitor efforts to control the spread of the pathogen.

Expected Outcomes:

1) Development of rapid, and reliable techniques for detecting and monitoring *Phytophthora* species.
2) Identification of *Phytophthora* species from native oak woodlands.
3) Technology transfer to user groups.

Implementation of Products/methods: The three principal investigators, in collaboration with Forest Health Protection, will lead the dissemination of information developed in publications, workshops, and websites. Statistically valid field surveys to determine the range of *Phytophthora* species using the developed techniques will be conducted by Forest Health Protection personnel.

PRODUCTS AND DUE DATES: 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.

PUBLICATIONS: Publications in refereed journals, University bulletins, and broader-based journals (e.g. California Agriculture).

TECHNOLOGY TRANSFER: A series of workshops will be held for State and County field and laboratory personnel, Forest Pest Management specialists, and private consultants. At these workshops, we will demonstrate *Phytophthora* isolation and identification techniques. Information on the design of surveys to detect *Phytophthora* in the field will also be presented. Information on isolation techniques will be posted on the University of California SOD Research website (http://camfer.cnr.berkeley.edu/oaks/).

LONG-TERM BUDGET REQUEST: *Note –for each requested STDP dollar category, the amount in the cell will be distributed 1/3 to OSU (Hansen), 1/3 to UCD (Rizzo), and 1/3 to UCB (Garbelotto).*

	Item	**Requested FHP STDP Funding**	**Other-Source Funding**	**Source**
FIRST YEAR				
Administration	Salary (3 Temp, grad student)	$54,000	$45,000 (0.1 FTE for each PI)	OSU, UCD, UCB
	Overhead	$0	Waived	OSU, UCD, UCB
	Travel	$3,000	$3,000	UC Integrated Hardwood & Range Mgt. Program
Procurements	Contracting	$0		
	Equipment	$0		
	Supplies	$9,000	$12,000	UC Integrated Hardwood & Range Mgt. Program
1st Year Totals		$66,000	$70,000	
SECOND YEAR				
Administration	Salary (3 Temp; grad student)	$54,000	$45,000 (0.1 FTE for each PI)	OSU, UCD, UCB
	Overhead	$0	Waived	OSU, UCD, UCB
	Travel	$3,000	$3,000	UC Integrated Hardwood & Range Mgt. Program
Procurements	Contracting	$0		
	Equipment	$0		
	Supplies	$9,000	$12,000	UC Integrated Hardwood & Range Mgt. Program
2nd Year Totals		$66,000	$60,000	
PROJECT TOTAL		**$132,000**	**$120,000**	

BENEFIT AND COST: Enclosure 2a.

STDP PRODUCTION FUNCTION

PROJECT NUMBER: R5-2001-01

PROJECT COST: $252,000

PROJECT OBJECTIVE:
The objectives of this project are to develop detection techniques (molecular diagnostic tools), baiting methodologies, and sampling procedures to determine extent of SOD *Phytophthora* and related species. The techniques, methodologies and procedures will be used to monitor effectiveness of control recommendations. Technologies developed by this project will enable rapid, accurate detection of oak pathogens, resulting in evaluation and possible modification of recommendations to slow the spread of the disease.

Without the project, pathogen detection will be inaccurate and unreliable, resulting in failure to correctly determine methods/time of year of spread, and failure to monitor spread. These failures will result in spread of the disease to currently uninfested areas, and in an increase in oak mortality. With the project, recommendations to slow the spread of the disease will be based on pathogen biology, and monitoring will be effective, resulting in a reduction of spread, and a reduction of oak mortality.

The measurable, quantifiable benefit analyzed here will be a reduction in tree removal and tree replacement costs. Tree removal expenses are quite high in urban situations where large oaks are adjacent to homes and other targets. Homeowners in Marin County are currently paying $1,000 - $2,000/tree for removal.

The primary benefit of this project, which is development of techniques that will slow the spread of oak mortality in unique ecosystems (in California and other states with oak resources) is intangible, and cannot be quantified here. The massive dieback of tanoaks and other oaks presently occurring will result in alterations to forest woodland ecology, with unknown and possible dramatic implications for wildlife habitat. A reliable method for monitoring the spread and control recommendations will reduce this effect. A second intangible, or at least difficult to quantify, benefit of this project is reduction of fuel loads in highly populated urban/wildland interfaces, resulting in reduced risk of catastrophic fire.

ASSUMPTIONS:

Without the Project:
- 1,000 urban landscape oaks will be killed over the next 5 years.
- 1,000 oaks will be removed, to eliminate hazard and fuel load.
- 500 oaks will be replaced, to maintain property value.

With the Project:
- Reduction in disease spread as a result of effective monitoring will reduce losses of urban landscape oaks by 60% over the next 5 years.
- 400 urban landscape oaks will be killed over the next 5 years.
- 400 oaks will be removed.
- 200 oaks will be replaced.

BACKGROUND DATA:

- Tree removal cost = $1,000 per tree.
- Tree replacement cost = $250 per tree.
- Treatment cost = $0 (voluntary washing of vehicles, equipment; seasonal closure of roads/trails).

CALCULATIONS

EXPENDITURE & OUTPUT VALUES (EOV) WITHOUT PROJECT:
- [(1,000 trees removed) ($1,000/tree)] + [(500 trees replaced) ($250/tree)] discounted for 5 years
= $1,000,000 + $125,000 = $1,125,000/1.22 = $922,131

EXPENDITURE & OUTPUT VALUES (EOV) WITH PROJECT:
- [(400 trees removed) ($1,000/tree)] + [(200 trees replaced) ($250/tree)] discounted for 5 years
= $400,000 + $50,000 = $450,000/1.22 = $368,852

BENEFIT (CHANGE IN EOV) ATTRIBUTABLE TO PROJECT:
B = $(without) - $(with) = $922,131 - $368,852 = $553,279 cost savings

BENEFIT/COST RATIO:
B/C = (Benefit Attributable to Project)/(STDP Cost + Contributed Costs) = $553,279/$252,000 = 2.2

BENEFIT ATTRIBUTABLE TO STDP FUNDS:
Benefit Attributable to STDP Funds = (STDP Cost) x (B/C) = $132,000 X 2.2 = $290,400

PNV OF PROJECT:
PNV of Project = (Benefit Attributable to Project) – (STDP Cost + Contributed Costs)
= $553,279 - ($132,000 + $120,000) = $553,279 - $252,000 = $301,279

PNV OF STDP:
PNV of STDP = (Benefit Attributable to STDP Funds) – (STDP Funds Requested)
= $290,400 - $132,000 = $158,400

LITERATAURE CITED/REFERENCES:

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