Enclosure 2

Special Technology Development Program

New Project Proposal

PROJECT NUMBER (Region-year-sequential number/priority: R5-2002-01

PROJECT TITLE: Development of methods to quantify insect born inoculum and the potential to incite new black stain root disease centers in eastside pine ecosystems.

PROJECT STATUS: New -not previously funded

EXPECTED PROJECT DURATION: Three Years

EXPECTED COMPLETION DATE OF THE PROJECT: 2005

SUBJECT:

1. Total Suppression/Prevention Technology	%		2. Survey and Monitoring Technology	%	
a. Total Biological Control i. Microbial % ii. Parasitoides % iii. Synthetic hormones/pheromones % iv. Other %		%	a. Advancements in Detection Technology		%
			b. Landscape Level Assessment Technology i. Data Visualization		%
b. Total Modeling i. Pesticide (Insecticide) Application % ii. Disturbance % iii. Growth and Yield % iv. Organism %**50** **v.** Population % **50** vi. Terrain %	100	%	c. Remote Sensing i. Aerial % ii. Hyperspectral % iii. Satellite %		%
			d. Other		%
c. Genetic, Cultural and Silvicultural Innovations For Controlling Pest Species i. Fire % ii. Methyl Bromide Alternatives % iii. Thinning/Regeneration Techniques and other Silvicultural Guidelines % iv. Resistance, Screening, and Breeding %		%	3. Assessment Technology	%	
			a. GIS % b. Spatial Analysis % c. Landscape Analysis % d. Decision Support % Risk and Hazard % Expert Systems %		
d. Pesticide Application (Spray) Technology i. Equipment innovations % ii. Methods and Guidelines %		%	4. Social Values	%	
			5. Technology Transfer Innovations	%	
c. Other		%	6. Other	%	

STATUS OF SUBJECT SPECIES: native

PROJECT OBJECTIVES:

1) Develop a reliable method to demonstrate and quantify inoculum potential carried by putative vectors of *Leptographium wageneri*.
2) Define stand and tree conditions contributing to new black-stain infection centers.

BRIEF DESCRIPTION OF PROJECT:

Year One:

- Stand selection (black stain infected)
- Pathogen isolation from infected roots
- Trapping configuration set-up
- Insect trapping and identification
- DNA Isolation and characterization

Year Two:

- Insect trapping
- Quantification and assessment of inoculum by insect species
- Tree vigor assessment
- Root inoculation

Year Three:

- Assessment of artificially induced infection.
- Construct conceptual model —re/ insect populations and proportion vectoring pathogen from DNA analyses, inoculum vs. tree / stand conditions.

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

Name	Affiliation (Office or Dept.)	Phone, E-mail, Fax
Sheri Smith	FHP, Lassen National Forest	530-252-6667 ssmith@fs.fed.us 530-252-6428

FHP LEAD INVOLVEMENT (add lines as necessary)**:**

Role	Time Commitment
Insect trapping, insect speciation, stand/site assessment	4 weeks

PRINCIPAL INVESTIGATOR(S):

Name	Affiliation (Office or Dept.)	Phone, E-mail, Fax
William J. Otrosina	Southern Research Station 320 Green St. Athens, GA 30677	706 559 4295 wotrosina@fs.fed.us 706-559-4291
Matteo Garbelotto	University of California	510 643 4282

Dept. ESPM
Hilgard Hall
Berkeley, CA

matteo@nature.berkeley.edu
510-643-5098`

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

Name	Role	Time Commitment
Otrosina	Inoculation studies, isolation, tree vigor assessment	50%
Garbelotto	DNA isolation, characterization	25%

COOPERATORS:

Name	Affiliation (Office or Dept.)	Phone, E-mail, Fax
Al Vazquez	Lassen NF	530-257-6428 avazquez@fs.fed.us 530-252-5803
Stanley J. Zarnoch	Southern Research Station Asheville, NC	828 259 0515 szarnoch@fs.fed.us

COOPERATOR INVOLVEMENT (add lines as necessary)**:**

Name	Role	Time Commitment
Vazquez	Site selection	5%
Zarnoch	Statistical design, analyses	5%

JUSTIFICATION (How does the project strengthen FHP program delivery/capability? What is the potential advantage over existing technology?)**:**

Currently, there is no direct scientific data demonstrating the vector relationship between root feeding bark beetles, weevils and certain other root inhabiting insects and black stain root disease. There is a strong body of circumstantial evidence for this vector relationship. Traditional means of determining vector relationships depend upon isolation of *L. wageneri* from the suspect insects. This has not been done due to limitations involved in isolating a slow growing fungus among a vast array of highly competitive contaminants present on insects. DNA techniques have been refined to a point where it is now feasible to develop probes specific to the taxon in question. Development of reliable probes would permit 1) provide demonstrative proof of vector relationships across insect species, 2) provide for study of ecological relationships between these insects, site disturbance, and disease transmission over distances, 3) from this information, enable evaluation of tree/stand condition relative to beetle populations and determine probability of new infections.

URGENCY (Does the project address a crisis situation? Would delay result in irreversible loss?)**:**

Currently, black stain root disease is becoming more prevalent in certain eastside pine stands. Mortality is rapid and presents problems not only related to loss of stems and growth, but to fuel/fire hazard creation as well. With wild fires being on the forefront concerning our national forests, information leading to the minimization of losses to this disease would serve to mitigate wildfire hazards in affected east-side pine stands. As with most disease processes, delaying efforts that contribute to disease mitigation will compound difficulties associated with managing black-stain root disease because of suspected insect involvement in its mode of spread

NATIONAL FHP TECHNOLOGY DEVELOPMENT PRIORITY (check at least one and describe how proposed work addresses the priorities):

Priority 1: __ Priority 2: __ Priority 3: _X_ Priority 4: __

Develop techniques to quantify the impact (forest structure and function) of pest species as disturbance agents in forest ecosystems including their interaction with other disturbance agents such as fire, and wind.

The proposed work relates directly to the disturbance component of the national priority 3. Knowledge of the insect vector relationships to incidence and impact of black stain root disease will lead to development of management guidelines and will be the logical next step to TDP R5-1999-01.

TECHNICAL COMMITTEE DEVELOPMENT PRIORITY (check at least one and describe how proposed work addresses the priorities): Technologies that detect, quantify, or predict the effects of pathogens and other microorganisms on resource production and ecological processes.

Priority 1: _X_ Priority 2: __ Priority 3: __ Priority 4: __

Priority 5: __ Priority 6: __ Priority 7: __ Priority 8: __

This project addresses priority 1 of the disease technical committee. Develop of a DNA probe to detect *Leptographium wageneri* on insect vectors and subsequent studies on insect population dynamics in relation to incidence of the root disease will lead to management recommendations to reduce the adverse impacts of the disease on resource production and ecological processes.

SCOPE OF APPLICATION (How widely are results likely to be applied—geographic area, range of pests, length of time?): Immediate application is to the eastside pine type, however, black stain root disease affects Douglas fir and pinyon as well. Also, the technology developed can become a model system applicable to other insect-disease complexes. For example, understanding stress relationships in trees and knowing probability of transmission and amount of inoculum to incite disease can have wide applicability to other root diseases such as annosus root disease, further refining the existing model.

RESEARCH BASIS (strength of research basis, including publication citations)**:** DNA technology has made exponential progress within the last five years (Garbelotto et al. 1996). Availability of new laboratory instrumentation and general understanding of genomic characteristics makes application of these techniques in ecological and pathological investigations possible. Also, new physiological evaluations of trees based upon biochemical responses contribute to the utility of the present proposal. (Sung et al. 1993, Otrosina et al. 1996).

METHODS (project design, hypothesis, statistical approach, and QA/QC procedures)**:**

1) Site selection and insect trapping — Sites having known black stain root disease will be selected to study insect transmission of the disease. At least four east-side ponderosa pine sites will be monitored for insect populations during the initial phase of this technology development project. Known sites currently used in related studies involving black-stain rot disease are located in the Modoc and the Lassen National Forests. Additional sites will be selected. Local isolates of the fungus will be obtained from infected trees in subject stands as reference cultures for DNA probe development. Initial insect trapping will involve the four study sites in order to obtain a wide variety of insect taxa. Trapping arrays will be initially deployed to maximize catch. Insects will be sorted by species and population, and will be preserved for DNA extraction and characterization. DNA techniques employed are standard procedures and taxon specific probes will be developed (Garbelotto et al. 1993, 1996). Once developed, these probes will be used to conduct surveys on insect catches from trap arrays deployed in year two and three. For this purpose, trap arrays will be designed to yield a random sample of root feeding insect species and their populations in order to assess inoculum distribution (both in the statistical and the biological sense) among sampled stands and insect taxa.

2) Determination disease potential — Little is known regarding how much inoculum of this pathogen and what the physiological state relative to stress is a necessary condition for development of disease in stands. Ascertaining inoculum density thresholds necessary for root colonization by *L. wageneri* will, when combined with knowledge gained from DNA assays for the pathogen on insects, provide the

means for developing a conceptual model strengthening management decisions making concerning at risk stands. To this end, pathological and physiological procedures designed to assess tree physiological status will be conducted on randomly selected trees within several stands. Selected trees will be screened using sucrose synthase activity as an indicator of stress (Otrosina et al., 1996; Sung, et al. 1993). These trees will be divided in two classes, normal activity and low activity. Large (> 10 cm) woody roots will be exposed from representative trees of each class and inoculated with spore suspensions containing known quantities of spores in three logarithmic quantity ranges, e.g., 100, 1000, and 10,000 spores/ml suspension. Exact concentrations will be determined via pilot inoculation studies in year one. Spore suspensions will be dispensed into wounds created by a cork borer or increment hammer into the outer xylem. Inoculations will be conducted early, middle, and late growing season mimicking root feeding insect flight patterns. Eight weeks after covering the inoculation site and roots, they will be re-excavated and lesions measured and excised for isolation of the pathogen and using DNA verification of pathogen presence. These data will allow conceptual understanding of what physiological conditions and what quantities of inoculum carried by these insects are necessary in order to incite disease.

Statistical analyses will be conducted on all inoculation and sucrose synthase data.

MEASURES OF SUCCESS:

Standard of Success: Development of DNA markers. Completion of physiological and pathological assessments.

Expected Outcomes:

1) Publishable results suitable for refereed journals.

2) Establishment of methods to assess potential risk of black-stain root disease. This would add value to other ongoing risk assessment based upon soils and vegetation data.

Implementation of Products/methods: Once developed, DNA technology can be implemented immediately as a tool for insect vector assessment. Physiological and pathological data will complete the conceptual model that associates insect data and tree / stand conditions to disease risk.

PRODUCTS AND DUE DATES:

End of FY 2002—DNA markers developed.

End of FY 2004—physiological and pathological data evaluated and draft conceptual model developed. Publications and reports completed by beginning of 2005.

PUBLICATIONS (how results will be reported: journals, reports)**:**

Refereed journals, Forest health bulletins.

TECHNOLOGY TRANSFER (Who will the technology be transferred to. How will products or methods be transferred to users, adapted to other uses, or sustained by continuing technology transfer?)**:**

Land management personnel will be the ultimate user. The technology will be transferred through personal contacts, published information.

PRODUCT LEVERAGING (Is the project part of a development sequence? Does it build on or is it the result of past Research or STDP projects?):

This project is the next logical step derived from two prior TD projects relating disturbance and stand management interactions with black stain root disease.

LONG-TERM BUDGET REQUEST: (estimates by fiscal year and funding, both monetary and in-kind, excluding FHP base funding and salaries) (extend table as necessary)**:**

	Item	Requested FHP STDP Funding	Other-Source Funding	Source
FY 2002				
Administration	Salary	$13,000	$ 75,000	Southern Research Station, Tree Root Biology
	Overhead	$ 5,550		
	Travel	$ 4,000		
Procurements	Contracting	$15,000 (DNA Analyses)		
	Equipment			
	Supplies	$ 5,000		
YEAR TOTALS		$ 42,550	$ 75,000	

FY 2003				
Administration	Salary	$13,500	$ 75,000	Southern Research Station, Tree Root Biology
	Overhead	$ 3,675		
	Travel	$ 5,000		
Procurements	Contracting			
	Equipment			
	Supplies	$ 6,000		
YEAR TOTALS		$28,175	$ 75,000	

FY 2004				
Administration	Salary	$14,000	$ 75,000	Southern Research Station, Tree Root Biology
	Overhead	$ 3,825		
	Travel	$ 5,500		
Procurements	Contracting			
	Equipment			
	Supplies	$ 6,000		
YEAR TOTALS		$29,325	$75,000	
PROJECT TOTALS		$100,050	$225,000	

LONG-TERM BUDGET REQUEST EXPLANATION: (add lines as necessary):

Contracting const ($15000) represents coop agreement with UC Berkeley for DNA Analyses. Other costs—travel, supplies, relate to pathological and physiological investigations. Salaries are for part time temporary technical support. The other source funding represents contributed salaries of Tree Root Biology scientists.

BENEFITS (What are the proposed benefits of this project): This project is the next logical step to the foundations laid by previous TD projects (R5-1999-01, R5-1993-01 and R6-00-03)). Development of a DNA probe and its use in insect vector population dynamics directly contributes to management of black-stain root disease through utilization of state of the art technologies. Principles learned from developing this technology have potential applications throughout the eastside pine type in California and Oregon, and toward other fire dependent ecosystems undergoing similar processes. Reductions in levels of black stain root disease incidence and impact as a result of management guidelines developed from this technology development will result in maintenance of fragile ecosystems. In addition to production of volume, the eastside pine type is heavily used by T&E wildlife species and sensitive wildlife species, including the bald eagle, goshawk, and others. A reduction of black stain root disease incidence will also lead to significant reduction of fuels present on the forest floor, and a reduced risk of catastrophic fire in the infested stands.

LITERATURE, CITATIONS, ATTACHMENTS, etc.:

Garbelotto, M., Ratcliff, A., Bruns, T.D., Cobb, F.W., and Otrosina, W.J. 1996. Use of taxon-specific competitive priming PCR to study host specificity, hybridization, and intergroup gene flow in intersterility groups of *Heterobasidion annosum. Phytopathology 86:543-551.*

Otrosina, W.J., Sung, S.S., and White, L.W. 1996. Effects of subsoiling on lateral roots, sucrose metabolizing enzymes, and soil ergosterol in two Jeffrey pine stands. Tree Physiology 18:1009-1013.

Sung, S.S., Kormanik, P.P., and Black, C.C. 1993. Vascular cambial sucrose metabolism and growth in loblolly pine in relation to transplanting stress. Tree Physiology 12:243-256.