LCMR Abstract

Project Title and Project Number: Biological Control of Overland Spread of Oak Wilt - 13(b)

Current measures to control overland spread of oak wilt range from efficient to haphazard. Where oaks with spore mats are not treated, they undermine community efforts to save healthy trees. This project was designed to test the application of a competitor fungus, *Ophiostoma piceae*, as a biocontrol agent to oaks producing or with a high potential to form spore mats in spring. A limited socio-economic, environmental, cost-benefit analysis compared the perceptions of property managers and property owners concerning such a biocontrol approach versus current practices (tree removal, log covering and girdling infected trees). *O. piceae* did not demonstrate sufficient competitive potential to serve as an effective control agent. However, the efficacy of spray application of fungal spores as developed for this research was effectively established facilitating the pursuit and development of other promising fungal antagonists. In the cost-benefit analysis, 64% of respondents considered oak wilt control as "very important" in their communities, cited lack of financial resources as the greatest obstacle to oak wilt control and cited numerous perceived advantages to using a biocontrol agent for disease control. Of 44 oak wilt managers surveyed, 32 felt that such naturally occurring biocontrol agents would make a difference in residents' attitudes about spraying. This research led to two related publications: Colonization of Oak Wilt Fungal Mats by *Ophiostoma piceae* During Spring in Minnesota, Plant Disease/Vol. 81 No.4; Acquisition of *Ophiostoma quercus* and *Ceratocycstis fagacearum* by Nitidulids from *O. quercus*-colonized oak Wilt Mats, Plant Disease/Vol. 82 No. 1.

Date of Report: December 31, 1997

LCMR FINAL REPORT

I. **Project Title and Project Number: Biological Control of Overland Spread of Oak Wilt -13(b)**

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A. **Legal Citation:** ML 95, Chp.220, Sec.19, Subd. 13(b)
Total biennial LCMR appropriation: $90,000
Balance: $0

Appropriation Language: This appropriation is from the future resources fund to the commissioner of agriculture in cooperation with the University of Minnesota to improve application methods for enhancing natural biological control of overland spread of oak wilt.

B. **LMIC Compatible Data Language:** Not Applicable.

C. **Status of Match Requirement:** Not Applicable.

II. **Project Summary:**

Current measures to control overland spread of oak wilt range from efficient to haphazard. Where oaks with spore mats present are not treated they undermine community efforts to save their healthy oaks. This project addresses this problem by augmenting levels of a naturally occurring rival fungus of oak wilt by spraying red oaks in which the oak wilt fungus has a high potential of forming spores in the spring (that is, hazard oaks). Our goal is to maintain a high level of the biocontrol fungus to reduce infectious material and overland spread.

The project will determine population levels of the naturally occurring competitor fungus (*Ophiostoma piceae*) in selected localities. It will survey population levels of *O. piceae* in locations (1) with long standing oak wilt control program and (2) with active oak wilt and no control program. The project will identify best management practices including optimal timing of applications, most efficient and effective equipment, best application sites and proper handling of *O. piceae* to maximize its effectiveness as a biocontrol agent. Finally, a limited socio-economic, environmental, cost-benefit analysis will compare biological control of the overland spread of oak wilt using *O. piceae* application versus current practices (girdling infected red oaks, removal and wood covering). Information sources for the analysis include interviews with current oak wilt managers, surveys of property owners and review of published and unpublished reports.

The goals of this project are to develop methodology and guidelines for operational application of the biocontrol fungus and to provide a decision making aid for communities and parks to use to determine best oak wilt control methods for their situation.

II. Six Month Work Program Update Summary

December 29, 1995: Accomplishments were made on all three objectives during the last six months:

Objective A: Samples of oak wilt fungus spore mats collected in the spring were processed to assess the naturally occurring population of the biocontrol fungus, *O. piceae*, present on the mats. This information gives us a baseline with which to compare the effectiveness of our augmentation sprays to increase the population of *O. piceae*.

Objective B: We conducted spray equipment trials to evaluate the ability of the equipment to provide adequate coverage of the stems and limbs of mature red oaks. If red oaks that die in the July and August are not treated, the oak wilt fungus can produce spores next spring under the bark of these trees. In addition, a set of trials was conducted to evaluate the viability of the biocontrol fungus when sprayed under pressure. A colleague found that many of the bacteria he sprayed as biocontrol agents to control weeds were killed by the high pressure spray equipment used. Our results using the high pressure spray equipment we selected were negative. The equipment does not harm our biocontrol fungus. Testing is also underway to develop procedures for producing *O. piceae* in quantity without the mucilaginous material that plugs spray nozzles.

Objective C: We developed a questionnaire and had it reviewed by several community foresters to get feedback about the relevance and clarity of the questions. Interviews of oak wilt managers begin in January.

July 1, 1996: Accomplishments.

Objective A: We completed analysis of the samples collected by March. The results indicate a range of coverage including little coverage of the immature spore mats and extensive coverage of most mats in the post-mature stage. A manuscript is submitted for publication in a refereed plant science journal.

Objective B: Spray equipment trials were completed in fall 1995. Biocontrol fungus inoculum (*O. piceae*) production methods have also been developed. We used the procedure to successfully produce inoculum without mucilaginous material for the spring 1996 operational spray trial.

We conducted a literature review on timing and peak oak wilt fungus spore production events. Factors such as bark surface and cambial temperature, sapwood moisture content, date of tree wilt, tree diameter, and north-south latitudinal location were monitored at north and south Twin Cities metro locations this spring. Staff also will collect data from July 1996 to June 1997. We will use key variables identified from that data to construct a model predicting when oak wilt sporulation will occur. This will help us time spray application of the *O.* piceae biocontrol fungus to maximize reduction of oak wilt spore production.

Objective C: We had a setback in the timeline of this objective. The questionnaire went through two separate revisions after preliminary interviews and other time commitments for

the staff doing the interviewing prevented full implementation of the survey this spring. The questionnaires will now be mailed out to oak wilt managers in October.

January 1, 1997: Accomplishments.

Objective A: We showed natural populations of the biocontrol fungus (*O. piceae*) colonized and covered more of *Ceratocystis fagacearum* (oak wilt) spore mats as the mats aged. We surveyed oak wilt spore mats for *O. piceae* in three locations. Subsamples yielding *O. piceae* ranged from 11 to 27% for immature spore mats, 65 to 72% for mature and 66 to 96% for aging and declining spore mats. Frequencies of *O. piceae* isolation from at least one subsample of an immature mat ranged from 30 to 53% compared to over 90% for all other ages of oak wilt spore mats. This objective was completed and the final report has been tentatively accepted for publication in a refereed plant science journal (see copy attached to end of update).

Objective B: Staff analyzed temperature data collected in spring 1996 from oak trees in Burnsville and North Oaks. They want to determine the best means to predict the period when oak wilt spore mat production peaks in the spring. In August 1996, 19 oak wilt killed red oaks in Blaine and Eagan were selected for spring 1997 monitoring. We will observe microsite temperature, sapwood moisture content and oak wilt spore mat production to refine the method for predicting the onset of sporulation.

Processing of the 2,898 subsamples collected during the spring 1996 operational spray trial took 5 months. Data summarization and analysis began in early December.

Over 50 red oaks killed by oak wilt in Dakota County's Lebanon Hills Regional Park last summer were marked for inclusion in the 1997 spring spray trials. Staff will monitor these trees in early spring and those with new oak wilt spore mats developing on them next spring will be candidates for the biocontrol treatment. We will spray treated trees with the *O. piceae* spore and water solution to reduce the overland spread of the oak wilt this spring.

Objective C: We contacted over 70 municipal and county oak wilt program managers last October to request their help on a survey of oak wilt program administration in Minnesota. Sixty-five agreed to participate and we sent copies of the Oak Wilt Questionnaire (see attached copy) to them. To date (late December), the cooperators completed and returned 39 copies of the survey. Staff will make a follow-up call to program directors who have not sent in their surveys in early January and then begin data summarization and analysis later that month.

June 30, 1997: Accomplishments.

Objective A: Final report published in peer-reviewed scientific journal (see attached paper).

Objective B: Predicting Peak Sporulation. Environmental and individual tree conditions related to oak wilt mat formation were monitored in two sites from early April through the end of June. A USDA Forest Service cooperator will use temperature data, sapwood moisture content data, and tree diameters as predictors in an effort to model peak oak wilt mat production during spring in East Central Minnesota. The USDA Forest Service has assumed this project as a second year of data (spring 1998) is needed to validate preliminary model(s) developed from the 1997 data.

Operational Spray Trial. The spring 1997 operational *O. piceae* spray trial was completed during the second half of May and involved 22 trees located in Lebannon Hills Park, Dakota County. The original objectives were revised based on findings of 1995 and 1996 field trials. Laboratory processing of study samples began in early June and will be completed by a USDA Forest Service cooperator.

Objective C: We received responses from 45 oak wilt program managers. Questionnaire responses were entered into a database, and analysis has begun.

December 31, 1997: Accomplishments and Final Report

Objective B: Through related cooperative studies of insect vectors visiting oak wilt mats on *O. piceae* sprayed trees we found that although the extent of *O. piceae* colonization and density of *O. piceae* propagules can be increased using augmentation sprays, biocontrol mediated by these insects is probably not occurring as reported in earlier scientific publications (see attached paper). However, we were able to use *O. piceae* as a marker and surrogate biocontrol organism in developing an effective delivery system for potential antagonistic fungi for biocontrol of oak wilt. Specifically, application of *O. piceae* using a commercially available, high pressure sprayer resulted in more extensive colonization and higher *O. piceae* propagule density on younger oak wilt mats on *O. piceae* sprayed trees compared to that on younger mats of untreated trees. Uniform delivery of *O. piceae* inoculum from 0 to 9 m was achieved. USDA Forest Service cooperators are currently pursuing development of other fungal antagonists for future operational spray trials that will utilize this delivery system.

Objective C: Surveys were collected from managers in all communities with oak wilt control programs. Results were analyzed, and a summary report prepared (see attached). Because the only effective measure for controlling the overland spread of oak wilt is tree removal, managers indicated that a biological control fungus that actually stopped overland spread would be a great addition to the oak wilt control tool box. They also said that it would be beneficial to property owners to be able to spray a tree to prevent overland spread while delaying the costs of tree removal.

IV. Statement of Objectives:

A. Assess natural populations of *O. piceae.* Survey population levels of *O. piceae* in locations (1) with long standing oak wilt control program and (2) with active oak wilt and no control program.

B. Develop environmental, cost effective methods for enhancing natural biological control of overland spread of oak wilt. Develop best application protocol including timing of applications, equipment, best application sites and handling of a natural biological control agent that can eliminate oak wilt fungus.

C. Conduct environmental and socio-economic cost-benefit analysis on *O. piceae* for oak wilt control and compare with current approaches to reduce sources of spores for the overland spread of oak wilt (removal and treatment of red oaks, stacking and covering red oak wood piles, girdling infected red oaks to facilitate drying of wood). Information sources include

interviews with current oak wilt managers and property owners and review of published and unpublished reports.

Timeline for Completion of Objectives:

	7/95	1/96	6/96	1/97	6/97	12/97
Objective A:						
Assess natural populations of *O. piceae*	xxxxxxxx		x (3/31/96)			
Objective B:						
Develop methods for biocontrol of overland spread of oak wilt	xxxxxxxx				x (6/30/97)	x (12/31/97)*
Objective C:						
Conduct cost-benefit analysis comparing current and biocontrol approaches			xxxxxxxx		x (6/30/97)	x (12/31/97)*

* LCMR extension to December 31, 1997 granted July 14, 1997

V. Objectives/Outcomes:

A. Assess natural populations of *O. piceae*

A.1. Activity: Process field samples and analyze data

A.1.a. Context within the project:

Baseline data on natural colonization is required to determine whether *O. piceae* augmentation sprays would benefit all oak wilt areas.

A.1.b. Methods:

Field samples collected during spring 1995 by USFS cooperator from a township with a high level of oak wilt and no organized control program will be processed. Field data will be recorded on each individual fungus mat collected. Information includes collection date, tree number, infection center location and mat development stage. In the laboratory ten subsamples (25 mm^2) will be taken from margins of each mat for serial dilution plating on acidified potato dextrose agar (PDA). Subsamples will be placed in separate vials and remain in frozen storage until processed. Isolation of *C. fagacearum* and *O. piceae* from each processed subsample based on colony-forming-units observed will be recorded for each subsample of each collected mat. All field and laboratory data will then be combined, stored and updated as needed in spreadsheets in electronic files. All files will be maintained on an IBM compatible PC and will be available for use on either IBM or Macintosh-type PC's. Colonization and fungal recovery data will be analyzed using standard ANOVA and categorical methods. Analysis and summarization will also include a similar dataset collected by USFS cooperators for a second area with long-standing oak wilt control program in 1994.

A.1.c. Materials:

- Expendable laboratory supplies (e.g. glassware, chemicals and agar for media, petri dishes) are required for this activity. Amount: $2,100
- An automatic dispensing system with appropriate pump and tubing and a vortex mixer will be needed for this activity as well as B.3; thus, cost will be shared across objectives. Amount: $1,200
- A PC-type computer, printer and software is required for data entry and analysis for this activity and for other activities under objectives B and C; thus, cost will be shared across objectives. Amount: $1,060.

Note: Dispensing system and computer would remain with the University of Minnesota at the end of the 2 years.

A.1.d. Budget:

Item	Amount	Expended $
1. salary and benefits		
- senior technician (22 wks.)	$11,880	11,880
- laboratory technician (14 wks.)	4,100	4,100
2. supplies	2,100	02,100
3. equipment	2,260	02.260
4. miscellaneous services (copying, statistical consulting, photography)	660	00,660
TOTAL:	$21,000	21,000

A.1.e. Timeline:

Product #1:
Written final report with executive summary on level of natural colonization of oak wilt spore mats by *O. piceae* in 1) an area with long standing oak wilt control program, and 2) an area with active oak wilt but no organized oak wilt control program: -- March 31, 1996

A.1.f. Workprogram Update

December 29, 1995 Data Report: Evaluation of Natural Colonization of Oak Wilt Mats by *Ophiostoma piceae*

Accomplishments: Oak wilt fungus mats were collected during spring 1995 from a municipality with low to moderate level of oak wilt control activity. LCMR funds were used to process subsamples taken from those mats and summarize collected data on the incidence of *O. piceae* colonization and the recovery of the oak wilt fungus, *Ceratocystis fagacearum* from the mats.

Data summary: Of the 15 immature mats collected, only one was totally covered by *O. piceae*. We found that 53% had no *O. piceae*, and 40% of the mats only had *O. piceae* on 10% of the mat surface. The mature mats showed a wide range of coverage. Over 75% of the post-mature mats were totally covered, while the remaining ones had > 10% coverage (see Table 1, below).

Table 1. Natural colonization of oak wilt fungus mats by *Ophiostoma piceae*, spring 1995, Ham Lake, MN

Development Stage	Number of trees producing the mats collected	No. mats collected	Number of mats with different levels of *O. piceae* coverage (%)										
			0	10	20	30	40	50	60	70	80	90	100
immature	8	15	8	6	0	0	0	0	0	0	0	0	1
mature	11	15	1	0	1	1	0	0	3	2	0	3	4
post-mature	13	30	0	1	1	0	1	0	0	1	2	1	23

July 1, 1996 Data Report: Evaluation of Natural Colonization of Oak Wilt Mats by *Ophiostoma piceae*

Accomplishments: Data analyses were completed in March 1996. A manuscript ("Natural Colonization of Oak Wilt Fungus Mats by *Ophistoma piceae*") is currently being written for submission to a refereed plant science journal.

January 1, 1997 Final Report: Evaluation of Natural Colonization of Oak Wilt Mats by *Ophiostoma piceae*

Accomplishments: Completed objective. Final report tentatively accepted for publication in refereed plant science journal (see attached copy). An abstract of the report is presented here. The natural colonization of different ages of *Ceratocystis fagacearum* (oak wilt) fungus spore mats by a biological control fungus (*Ophiostoma piceae*) on *Quercus* spp. (oak trees) was determined in three east central Minnesota locations during spring 1995. Extent of mat area colonized by *O. piceae* generally increased with age. Average percentages of subsamples per mat yielding the fungus for all locations ranged from 11 to 27% for immature, 65 to 72% for mature and 66 to 96% for aging and declining mats. Frequencies of *O. piceae* isolation from at least one subsample of an immature mat ranged from 30 to 53% compared to over 90% from all other mat ages. Average number of colony forming units of *O. piceae* per cm^2 ranged from 3×10^3 to 3×10^6 and generally increased with mat age. Extent of *C. fagacearum* recovery from each mat was lower for aging and declining mats ($P<0.03$) compared to other ages in two locations. Frequency of *C. fagacearum* recovery from at least one subsample of a mat averaged 99% for all ages of mats in all locations.

July 1, 1997 Final Report: Provided in January 1, 1997 Quarterly Update (see above) The resulting journal article is attached to this Final Report.

B. Develop environmental, cost effective methods for enhancing natural biological control of overland spread of oak wilt

B.1. Activity: Evaluation of spray equipment

B.1.a. Context within the project:

Experimental trials with augmentation sprays of *O. piceae* were conducted with hand-held sprayers. Higher capacity, commercially available spray equipment will be required for operational-scale augmentation sprays.

B.1.b. Methods:

Application equipment for operational application of *O. piceae* must give good coverage of tree bark with liquid, inoculum suspension; good mixing within tank; allow suspension to reach approximately 50 feet (height); be portable enough to transport to off-road, wooded sites; and be available to municipalities, parks, etc., for use. A brief survey of spray equipment (and their specifications) currently owned and used by municipalities and parks in southeastern Minnesota will be conducted. A quick review of other commercially available spray rigs that may also meet requirements, but are not currently used within the area, will also be conducted. Information resulting from the equipment survey will be summarized in a tabular format using a word processing program and stored as an electronic file accessible on either an IBM compatible or Macintosh-type PC. Standard protocols in application technology for assessing spray coverage and amount of drift will be used in field evaluating the most promising equipment. It is anticipated that aqueous dye solutions and assessment cards systematically placed at different heights on trunks of oaks will be utilized in these screening trials. Size of droplets deposited and density of droplet coverage on each card will be recorded using optical scanning and a computer program. All electronically collected data will be stored in a spreadsheet format. A 10% random sample of all cards will be selected for QA/QC. Droplet size and density will be assessed manually for these particular cards and results compared with the computer system. Four replicated spray treatments (i.e. four separate trees per treatment) will be included with each selected piece of equipment. These trials will be conducted in a Hennepin County park. ANOVA and comparison of means when significance is found will be used to analyze these data.

B.1.c. Materials:

- Expendable supplies (e.g. spray assessment cards, dye solution, tools, safety gear, etc.).
Amount: $300.

- Equipment:
    * Rental of spray application equipment and associated transport charges.

Amount: $1,000.
* PC-type computer system, printer and software purchase (see A.1.c.).
Amount: $800.

B.1.d. Budget:

Item	Amount	Expended $
1. Salary and benefits - senior technician (10 wks)	$5,400	5,400
2. Supplies (expendable)	300	300
3. Equipment (rental and purchase)	1,800	1,800
4. Travel	100	100
5. Miscellaneous services (copying, photography, telephone/fax charges, etc.)	100	100
TOTAL:	$7,700	7,700

B.1.e. Timeline:

Product #1:
Progress report with executive summary identifying equipment determined through inquiries and technical reports to be most promising for augmentation sprays and results of dye solution trials: -- March 15, 1996

B.1.f. Workprogram Update

December 29, 1995 Data Report: Sprayer Equipment Trials

Accomplishments: Three types of spray systems were evaluated in September 1995 for their ability to consistently place and adequately cover the bark of main stems and limbs of recently killed red oaks with water up to 50 feet in height. The systems tested included: a hand-held, mechanically operated slide sprayer; an ATV mounted sprayer with an electrically powered pump; and a truck mounted sprayer with a gasoline powered pump. Water sensitive cards were placed at 5 foot intervals between 15 and 50 feet prior to spraying; extent of coverage by the sprayed water on these cards was determined following treatment. The slide sprayer provided reliable, consistent coverage only to 20 feet. The ATV mounted sprayer placed material up to 35 feet, but consistent coverage occurred only to 30 feet. The truck mounted spray system delivered material to 45 feet with consistent coverage to 40 feet. Based on these results, we plan to use the later system for spring 1996 operational trials with *Ophiostoma piceae*. Additional testing found that the high pressures (450 psi) in the spray lines of the truck sprayer did not significantly affect the biocontrol organism's viability.

July 1, 1996 Data Report: Sprayer Equipment Trials

Accomplishments: Completed by December1995 -- See December 29, 1995 update.

B.2. Activity: Microbial application

B.2.a. Context within the project:

Experimental trials with augmentation sprays of *O. piceae* were conducted with laboratory-grown liquid-shake cultures of the fungus that were simply diluted to a certain concentration and applied to hazard trees (in which the oak wilt fungus has a potential to produce spores in the spring). Refinement of the inoculum production system, timing of treatment, and selection criteria for oak wilt areas likely to benefit from augmentation sprays are needed for an effective, operational spray program.

B.2.b. Methods:

Two approaches will be taken to refine the current *O. piceae* production system for operational-scale spray program. A literature review will be conducted to assess approaches used in development of other biocontrol micro-organisms. Depending on equipment requirements, one or more of these methods would be tested in the laboratory with *O. piceae*. Processes involving steps such as macropore filtration, centrifugation and lyophilization would be possible in our laboratories. The short-term goal would be to improve current inoculum production to significantly reduce or eliminate mucilaginous material in the inoculum; this material clogs spray nozzles. Minimal data collection is required for this activity. Secondly, companies involved in the manufacture of biocontrol fungal agents would be contacted as to their interest in commercially producing an *O. piceae* product. The ultimate goal is a commercial product that can be stored easily yet retains viability over a reasonable period of time; mixes well with an aqueous solution; flows well and evenly through spray apparatus; and provides some protection from UV degradation, general dessication and nutritional depletion.

Timing of spring applications of *O. piceae* in experimental trials has been based on approximate date of tree wilt the previous summer, tree cambium condition in early spring, and first observation of sporulation in the area. Latitude of spray location and tree diameter may also be helpful in predicting onset of sporulation during the critical spring period, in addition to environmental data.

Much data has been collected and summarized on mat formation in the past 40 years; these could be utilized to test a refined system for predicting sporulation onset. Simple predictive models will be constructed and tested with such available datasets. All datasets used would be stored as electronic files (spreadsheet format). Data analyses would be conducted with a commercially available statistics package (e.g., SYSTAT). QA/QC would involve testing the predictive model with datasets obtained during Objective A activities. Our aim is to apply *O. piceae* shortly after sporulation onset but prior to significant increases in amount of sporulation in a particular location.

Finally, results of Objective A studies coupled with the above results will be used to develop broad guidelines for assessing whether or not an area would likely benefit from *O. piceae* application. No numerical data collection is required for this activity.

B.2.c. Materials:

- Expendable laboratory supplies (e.g. chemicals and agar for media, petri dishes, flasks for liquid culture, centrifuge tubes, etc.).
  Amount: $500.
- Equipment - no equipment purchase or rental required for this activity.

B.2.d. Budget:

Item	Amount	Expended $
1. Salary		
senior technician (8 wks)	$4,320	4,320
2. Supplies	500	500
3. Equipment	0	0
4. Miscellaneous services (copying, photographic work, etc.)	100	100
TOTAL:	$4,920	4,920

B.2.e. Timeline:

Product #2:
Progress report with executive summary with written guidelines for *O. piceae* production and application including timing and sites: -- June 30, 1997

B.2.f. Workprogram Update:

December 29, 1995 Data Report: Inoculum Production Methodology

Accomplishments: Protocol for producing *O. piceae* inoculum without mucilaginous material that plugs up spray nozzles and clogs sprayer lines is currently being tested. Protocol developed and tested thus far includes: increasing fungal spores in liquid nutrient medium, vacuum filtration of resultant fungal growth, low-speed centrifugation (2,400 rpm), and suspending pelleted spores in an aqueous medium. Viability of inoculum is then tested. One experimental run with this protocol using two different culture media has been completed to date. No differences were found between the number of spores produced in the malt extract broth (5.32×10^7 spores/ml) and the potato extract broth (3.55×10^7 spores/ml).

July 1, 1996 Final Report: Inoculum Production Methodology

Accomplishments: Adequate inoculum levels (10^6 spores/ml) can be produced quickly (2-3 days). Simply filtering the inoculum through coarse filter cloth (Miracloth™) under vacuum is an effective and efficient procedure to produce inoculum without mucilaginous material. We used this method to produce 400 gallons of *O. piceae* inoculum for the spring 1996 operational spray trial.

July 1, 1996 Data Report: Predicting Peak Oak Wilt Spore Mat Production Period(s) in Spring

Accomplishments: We conducted a broad-based literature review on timing and conditions associated with peak oak wilt fungal sporulation events. We combined this knowledge with specific information available on oak wilt sporulation during the critical spring period. The first sporulation peak for the oak wilt fungus appeared to be environmentally triggered (primarily by temperature), based on our recent observations (1994-1996). We believe the major variables are: temperature, sapwood moisture content, date of tree wilt, and tree diameter. We also observed north-south latitudinal differences. We used ambient air temperatures recorded within oak stands or within several miles of the study site to generate temperature data available with existing datasets on oak wilt sporulation. We believe microsite temperature would be a better predictor. We decided to monitor both bark surface and cambial temperatures on recently killed red oaks in two locations (North Oaks and Burnsville, Minnesota) from mid-April to June 27, 1996.

The oak wilt fungus does not sporulate until sapwood in red oaks dries to a moisture content of 37 to 45 percent. The length of time needed for infected, standing red oaks to dry to this point is not available in existing datasets. Thus, we plan to collect this data between July 1996 and June 1997. We will use key variables identified from that data to construct a model predicting when oak wilt fungus spore mat peak production will occur. This will help us time spray application of the *O.* piceae biocontrol fungus to maximize reduction of oak wilt spore production.

January 1, 1997 Data Report: Predicting Peak Oak Wilt Spore Mat Production Period(s) in Spring

Accomplishments: Temperature data collected in spring 1996 from five oak trees in two locations (Burnsville and North Oaks) were analyzed to determine the best means to monitor this parameter. Tree aspect (north and south), height on tree (1 foot vs. 10 feet), and microsite for temperature measurement (inside bark = cambial vs. bark surface = ambient air) were considered. Our preliminary results found: 1) ambient air temperature to be a good measure, although it was not necessarily constant over the season, 2) the upper height was the better position for sensor placement, and 3) little difference was found for tree aspect, but the southern aspect is preferred for biological reasons. In August 1996, 19 oak wilt-killed trees in Blaine and Eagan were selected for spring 1997 monitoring that would include microsite temperature, sapwood moisture content and oak wilt spore mat production.

June 30, 1997 Final Report: Predicting Peak Sporulation Spring 1997

Accomplishments: Four study sites were established in early April 1997 (two in Blaine and two in Eagan, MN). Daily cambial temperatures were monitored between 2 April and 30 June on 19 trees on these sites. Sapwood moisture contents before, during, and after oak wilt mat production were also determined for the same trees. Weekly visual monitoring of these trees was also conducted and occurrence of fungal mats on each tree was recorded. Data summarization will begin in Fall 1997. Data analyses and preliminary modelling will be conducted by USDA Forest Service cooperators who have assumed responsibility for this continuing project. Additional data will be collected during spring 1998 for validation of preliminary model arising from the 1997 study.

B.3. Activity: Operational Spray Trials

B.3.a. Context within the project:

The main thrust of this project is to accelerate development of operational scale application of *O. piceae* to augment natural biocontrol of overland spread of oak wilt. This activity is the culmination of all previous activities; results of A.1, B.1 and B.2 will be used in designing and conducting operational-scale spray trials in spring 1996 and 1997 with *O. piceae*.

B.3.b. Methods:

Candidate oak wilt areas will be selected on the basis of: 1) type of area that will benefit greatest from augmentation sprays (result of A.1), 2) willingness of township, municipality, park, etc., to participate in operational trial (e.g. to provide candidate trees, access for spraying and evaluation), and 3) availability of sufficient number of trees in appropriate cambial condition for the trial. For each year, it is anticipated that 45 - 70 trees will be treated with *O. piceae* and approximately 15 - 20 trees will be required for control treatment.

O. piceae inoculum will be produced according to protocol developed in activity B.2. Timing of application will be primarily based on results of analyses conducted in B2; however, equipment availability and access may influence exact date of application. A known concentration of *O. piceae* will be mixed in the tank of the selected sprayer (result of B.1.) and suspension applied to achieve run-off to maximum 50 foot height depending on individual tree height and main stem diameter at top of tree. QA/QC activity will involve attaching open petri dishes with solid agar medium at different heights on study trees prior to treatment: plates will then be removed and covered immediately after spraying. The number of colony-forming-units will be recorded and compared to 1) microscopically determined inoculum concentration for the spray mix, and 2) results of assessment cards (from B.1.a.) for the particular application equipment used. Data on weather conditions at time of spraying will be recorded. Water will be used for treatment of control trees. All manually recorded field data will be subsequently entered into a PC and stored electronically.

Oak wilt fungus mats produced on *O. piceae* treated and control trees will be assessed weekly. Mats will be collected from the trees at different heights during the 4 weeks

following treatment. Assessment, sample processing, data analysis and QA/QC will be as described previously in section A.3.

B.3.c. Materials:

- Expendable laboratory and field supplies (e.g. glassware, pipettes, chemicals and agar for media, petri dishes, field sampling equipment, etc.) are required for this activity.
  Amount: $ 1700 per FY (season).
- Equipment rental and associated transport charges:
  * for spray application equipment - 2 - 3 days in spring 1996 and in spring 1997.
  * hi-ranger ("bucket truck") for sample collection during spring 1996 and 1997
  Total amount estimated = $1,100 per each spring.
- Contribution from this activity to PC-type computer purchase (see A.1.c. and B.1.a.) and toward purchase of automatic dispensing system (see A.1.c.).
  Amount = $2,600.

B.3.d. Budget:

Item	Amount	Expended $
1. Salary and benefits		
senior technician (49 wks - FY 1 and 2)	$27,200	27,200
seasonal field/lab technician (26 wks - FY 1 and 2)	10,280	10,280
2. Supplies (two season's work)	3,400	3,400
3. Equipment - rental and purchase	4,800	4,800
4. Travel	200	200
5. Miscellaneous services (phone/fax, photographic, statistical consulting, copying, etc.)	500	500
TOTAL:	$46,380	46,380

B.3.e. Timeline:

Product #3:
Final report with delivery system protocol for operational, augmentation sprays with *O. piceae* including: 1) recommended equipment, 2) timing of sprays, 3) inoculum production and 4) appropriateness of areas for treatment. Research trial results supporting this protocol would be in the appendix. -- June 30, 1997

Note: Ideally the due date should be September 30, 1997 to give time for processing 1997 spray trial samples, analyzing data and including this in the appendix of the report.

B.3.f. Workprogram Update:

July 1, 1996 Data Report: Spring 1996 Operational Spray Trial

Accomplishments: In April, we selected red oaks killed by oak wilt during summer 1995 for the operational trial. These were red oaks on which the oak wilt fungus had a high probability of forming spores this spring. They were located on private properties in the city of Ham Lake and in Columbus Township, MN. We produced biocontrol spray inoculum according to protocol developed in B.2. A high-pressure, truck-mounted sprayer with a hand-held spray gun applied the *O. piceae* and water mixture to 19 trees on May 14 and 16, 1996. The second application was necessary because heavy rain fell within a few hours of the first application. We were concerned the heavy rainfall would wash the biocontrol (*O. piceae*) spores off treated trees. The sprayer was able to spray to a height of 50 feet. Twenty additional trees were not treated. They serve as controls with which we compare the results of the spraying the biocontrol fungus on the treated trees. One and two weeks after treatment, we collected approximately 500 oak wilt spore mats from sprayed and control trees. We took small pieces of the spore mats (subsamples) from each of the spore mats collected. These are stored at -2° C until they can be processed. Processing of the 2700+ subsamples using dilution plating techniques began in June.

July 1, 1996 Data Report: Spring 1996/97 Operational Spray Trial

Accomplishments: Six subsamples from each of 483 spore mats were collected in spring 1996 for spray efficacy evaluation. They were processed in the laboratory using serial dilution plating techniques. Processing of the 2,898 subsamples required 5 months. Results on isolation of the oak wilt fungus and the competitor (*O. piceae*) fungus were recorded electronically. Data summarization and analyses began in early December 1996.

Permission was obtained to conduct the spring 1997 operational spray trial in Lebanon Hills Regional Park, Dakota County. Over 50 candidate red oak trees that were killed by oak wilt in summer 1996 were selected and marked during August and September for inclusion in the spring 1997 spray trials.

June 30, 1997 Data Report: Spring 1997 Operational Spray Trial

Accomplishments: Twenty-two red oaks killed by oak wilt during summer 1996 in Lebannon Hills County Park, Eagan, were used for the spring 1997 operational spray trial. *O. piceae* inoculum was produced according to the protocol developed in B.2. A high pressure, truck-mounted sprayer was used to apply the *O. piceae* mixture to 12 trees on 16 and 22 May. The second application was needed because several of the trees did not begin mat production until the later date and because new mats were continuing to be produced on the other trees. Ten additional trees were not sprayed and served as controls. Seven days after each spray date, oak wilt spore mats were collected from the study trees. Over 200 mats were collected for evaluation. Subsamples of the mats were removed for subsequent laboratory processing to evaluate treatment efficacy. Processing of the 1200 + subsamples using dilution plating techniques began 2 June and will be completed by 29 August. Data summarization, data analyses, and final report will be completed between 1 September and 31 December 1997.

December 31, 1997 Final Report: Spring Operational Spray Trials

Through related cooperative studies of insect vectors visiting oak wilt mats on *O. piceae* sprayed trees we found that although the extent of *O. piceae* colonization and density of *O. piceae* propagules can be increased using augmentation sprays, biocontrol mediated by these insects is probably not occurring as reported in earlier scientific publications (see attached paper). Objectives for the 1997 operational spray trial were accordingly revised based on findings of these insect-fungal acquistion studies. Specifically, *O. piceae* was used as a marker and surrogate biocontrol organism to determine if a high-pressure, commercially available, truck-mounted sprayer would deliver viable propagules of *O. piceae* to exposed oak wilt mats from 0 to 9 m height on recently killed trees and result in: a) significantly greater extent of oak wilt mat coverage by *O. piceae* for mats on trees receiving the treatment, and b) significantly greater number of viable *O. piceae* propagules per square centimeter of mat surface on mats formed on treated trees compared to non-treated ones.

In order for a biocontrol candidate to be successful in preventing acquisition of viable *C. fagacearum* spores from oak wilt mats, a biocontrol organism would have to arrive on a mat at as early a mat developmental stage as possible and quickly colonize the mat surface. In general, the results from the two operational spray trials demonstrate that the spray application resulted in a higher proportion of mat subsamples from younger mats yielding *O. piceae* compared to that of mats from untreated trees (see attached Tables 1 and 2). For seven of eight cases, no difference in extent of colonization was found when collection height of mats was considered. Thus, the sprayer was able to deliver viable inoculum equally to lower (0 to 4 m) and higher (4 to 9 m) heights of study trees.

The average number of propagules of *O. piceae* isolated per square centimeter increased with mat age ($P < 0.05$) (see attached Table 3). Differences in propagule numbers were found for the younger Ham Lake mats and for both the younger and older mats in the Eagan spray trial. Specifically, the numbers of colony-forming units varied by treatment ($P < 0.042$) in all three cases, and by collection height ($P < 0.03$) in only one case (older, Eagan mats). In particular, younger oak wilt mats on sprayed trees yielded higher numbers of viable propagules per square centimeter compared to that found for younger mats on untreated trees.

In summary, application of *O. piceae* using a commercially available, high-pressure sprayer resulted in more extensive colonization and higher propagule density of *O. piceae* on younger mats on sprayed trees compared to those on untreated trees. Uniform deliver of *O. piceae* inoculum from 0 to 9 m height was also found. These study results have demonstrated an effective delivery system for potential antagonistic fungi for biocontrol of oak wilt mats. Related studies are continuing by USDA Forest Service cooperators on identification and development of such biocontrol fungi.

C. Conduct environmental and socio-economic cost-benefit analysis on *O. piceae* for oak wilt control and compare with current approaches

C.1. Activity

C.1.a. Context within the project:

The knowledge gained will help oak wilt managers select and use most environmentally and economically effective management practices to control the overland spread of oak

wilt in their jurisdictional areas. Current efforts to control oak wilt include activities to stop the underground spread of the disease and to educate the public about the importance of not injuring oak trees, and removing potential spring spore producing (hazardous) northern red oak (*Quercus rubra*) and northern pin oak (*Quercus ellipsoidalis*) trees and treating the logs in the spring (April 15 to July 1) to prevent overland spread. Operations to isolate oak wilt infected trees from nearby healthy trees involves severing the root connections (install root graft barriers). When properly installed between diseased and healthy trees, root graft barriers stop the underground spread of oak wilt. Some jurisdictions practice these activities in a well orchestrated and efficient manner and in others they are scattered or seldom performed. Treatment of *Q. rubra* and *Q. ellipsoidalis* (red oaks) to prevent overland spread of oak wilt typically involves prompt removal of the trees and covering the stacked wood under plastic tarps from April 15 to July 1. Compliance with these procedures varies depending on the ability of the jurisdiction to educate and monitor these activities. Where red oak trees or logs are not treated, the oak wilt fungus can produce spores for the overland spread of oak wilt under the bark of these trees.

When *O. piceae* is applied to red oak at the proper time, it prevents formation of spores by the oak wilt fungus. We anticipate that this biocontrol method will be more effective than the standard treatment because it can be applied throughout a jurisdiction without having to worry about the lack of property owner follow through sabotaging the effort. It gives property owners options and time for removal and disposal of dead trees. We expect it is less costly with better accountability for the municipality or county. The biocontrol method has to be perceived as more beneficial or less costly than the current methods and adopted by oak wilt managers.

C.1.b. Methods:

An analysis of the ecological, social and economic costs and benefits of using *O. piceae* will be based on information obtained during interviews with oak wilt managers and property owners, and on reviews of published and unpublished annual budgets and reports about oak wilt control activities.

The cost analysis includes a comparison of three methods of treating hazardous red oak trees to prevent the overland spread of oak wilt:

1) removal of all oak wilt-killed red oak, stack and cover with plastic from 4-15 to 7-1

2) February or March inspection, removal of only potential spring spore producing red oak, stack and cover with plastic from 4-15 to 7-1

3) augmentation spraying of the biocontrol fungus, *O. piceae*; (application times and methods determined in Objective B).

We will use cost records from annual budgets and reports to analyze the cost of control methods 1) and 2) in the thirty-five to forty counties and municipalities actively controlling oak wilt.. We will estimate cost of reducing overland spread of oak wilt by enhancing natural populations of *O. piceae*. This will be estimated using data derived from field trials described in objective B. The field trials will be designed to be of a scale to give sufficient data for the comparison.

To determine the benefits of the methods, we will survey oak wilt managers in active jurisdictions. Questions covered in the survey include:

a) What is the benefit of controlling oak wilt?
b) Do you think oak wilt control is important? Rate on a scale of 1 to 10.
c) If you see it as important, how successful have you been with convincing property owners to comply with it and local politicians to support it?
d) If not successful, what are the barriers to implementing an effective program?
e) Why did it sell or what are the barriers to it selling in their jurisdiction?
f) What were the chief selling points or what were the biggest barriers?

We will analyze the information obtained from the first questionnaire, and identify factors that are important to adoption and successful application of an oak wilt control method (for example, lower costs, guaranteed success, perceived as environmentally safe by residents, leave the responsibility for follow-through in the hands of the property owner). We will then go back to the managers with our list of factors, and ask them to rate the importance of the various factors and to rate how well the current and the biocontrol methods do in addressing each factor. We will then know the relative benefits of each method.

C.1.c. Materials:

Supplies (notepads, diskettes, audio recorder, audiocassettes, *etc.*) and services (photocopying, phone, *etc.*) are required for this activity. Staff will use PC-type computer (see A.1.c and B.1.a) to compile data and write up report.

C.1.d. Budget:

Item	Amount	Expended $
1. Salary and Benefits		
Senior technician or research assistant (Two 16-week periods: 11/95-2/96 and 11/96-2/97)	$9,400	9,400
2. Miscellaneous supplies and services	300	300
3. Travel	300	300
TOTAL:	$10,000	10,000

C.1.e. Timeline:

Product #1

Progress report classifying relative benefits of three methods of controlling overland spread of oak wilt using initial survey interviews with oak wilt managers in municipalities and counties that actively address oak wilt: -- 12-31-95 and
prepare follow up questionnaire, distribute it to oak wilt managers and deliver report on evaluation of results: -- 12-31-96

Product #2

Report of data compiled from annual budgets: -- 2-29-96 and
compare the dollar cost of the two approaches: -- 2-28-97

Product #3
Final Analysis/Report for Oak Wilt Managers to use to select and use the most effective management practices to control overland spread of oak wilt: -- 6-30-97

C.1.f. Workprogram Update:

December 29, 1995 Data Report: Analysis of the ecological, social and economic costs and benefits of using *O. piceae*

Accomplishments: We developed a questionnaire and had it reviewed by several community foresters to get feedback about the relevance and clarity of the questions. Interviews of oak wilt managers begin in January.

July 1, 1996 Data Report: Analysis of the ecological, social and economic costs and benefits of using *O. piceae*

Accomplishments: We personally interviewed managers of five oak wilt programs (Apple Valley, Eagan, Sherburne County, Ham Lake and Columbus Township) in January using the questionnaire developed in December 1995. The managers made several suggestions to clarify the wording, broaden the scope and make the questions about control activities more specific. We revised the questionnaire in response to these suggestions.

In April we interviewed four more oak wilt managers (Lino Lakes, Chisago County, Isanti County and Blaine) with the revised questionnaire. Additional suggestions came from these interviews, including the advice to wait until fall to send out the formal questionnaire to all the oak wilt managers in Minnesota. They will be very busy in the spring and summer. We completed the final revisions in May.

We attached a copy of the questionnaire to the end of this Workprogram Update. Response to the questionnaire has been quite positive. We anticipate cooperation from a high percentage of the oak wilt managers receiving it in the fall.

January 1, 1997 Data Report: Analysis of the ecological, social and economic costs and benefits of using *O. piceae*

Accomplishments: We contacted over 70 municipal and county oak wilt program managers in October 1997. Sixty-five agreed to participate in a survey of oak wilt administration in Minnesota. We sent a copy of the Oak Wilt Questionnaire to them in early November (Please note: last update I said I was going to attach the Questionnaire and I forgot to do it. I will attach a copy to this update). To date (late December), 39 (60%) of the program managers completed and returned their surveys. Staff will make a follow-up call to those who have not sent in their surveys by early January and then begin data summarization and analysis later that month.

June 30, 1997 Data Report: Nothing to report at this time.

December 31, 1997 Final Report

Specification of products #1, #2, and #3 assumed that *O. piceae* would be effective in preventing the spread of the oak wilt fungus; it was not. Therefore, we surveyed oak wilt control program managers to determine the costs and benefits of control measures currently being used, and the potential costs and benefits of spraying a naturally occurring fungus to prevent overland spread. The questionnaire and a report of findings are included in an attachment to this report. If a biological control is found that stops the overland spread of oak wilt, findings from our survey would offer insights into the possible benefits and costs of this treatment:

- Oak wilt was the most common response to the question: "What are some of the major tree health issues facing your community?" More than 64 percent of the program managers said that the control of oak wilt is a very important tree health issue.

- Many managers indicated that their oak trees/stands are in pockets in parks and yards. When oaks are scattered, the threat of overland spread is greater than other means of transmission. A spray would offer a control option for keeping oak wilt infections confined to isolated areas.

- Current control efforts center on root trenching and tree removal. Many communities indicated that the greatest disadvantage of their current approach is that it does not control overland spread. Root trenching does nothing to prevent the spread of the disease to stands that are separated geographically. Tree removal, if done immediately and properly, will prevent overland spread, but if by spraying a naturally occuring fungus overland spread could be controlled and tree removal could be delayed, that would be a great benefit to property owners.

- Costs of current control efforts are high, and a large number of communities indicated that high costs of tree removal and root trenching are the major barriers to local government and private landowner support for the oak wilt control program. Root trenching averages $475/tree, with a range of $100 to $750 (see attached Table 4). Tree removal averages $343, with a range of $100 to $600.

- Communities indicated that oak wilt control on private land is covered primarily by the private landowner—on average, 67 percent of the costs are covered by the landowner. In general, private landowners comply with the steps outlined for control, with full compliance being achieved 71 percent of the time.

- When asked to evaluate the possible advantages of spraying a naturally occurring fungus to control overland spread of oak wilt, managers mentioned that dead trees could be left standing, that the spray would help limit removal costs, that there would be less cost to the homeowner, and that the treatment would be more cost-efficient than current methods. Disadvantages of spraying were that dead trees left standing could be a hazard, that dead trees lack aesthetic value, and possible opposition by environmental groups. Communities indicated that people may have a negative perception of spraying programs.

- Managers said that it would make a difference to residents if they knew that the mixture being sprayed was a naturally occurring biological control fungus—that it would receive higher acceptance than the spraying of an artificial substance. Public education would be needed before the implementation of such a control program, with the most effective approaches being public service announcements on television, radio, and in newspapers, and public meetings and demonstrations.

VI. Evaluation:

The project will be considered a success when the best methodology for operational application of *O. piceae* to oak wilt-killed red oaks has been identified and a decision making aid developed for communities and parks to use to determine the best oak wilt control methods for their situation. The data collected for this project will be part of the process to obtain US Environmental Protection Agency (EPA) registration. It is doubtful the product will be fully registered by EPA and commercially available by June 1997, but the process will be well on its way to completion.

For the first time in oak wilt control history a tool will be available that has the potential to approach 100% control of the overland spread of oak wilt in treated areas.

II. Context within Field:

University of Minnesota research developed methods of stopping the underground spread of oak wilt by cutting root connections between oak wilt infected and healthy oaks. Further research at U of M by Dr. David French and associates identified *Ophiostoma piceae* as a fungus that inhibited the ability of the oak wilt fungus to grow and produce spores, and protect fresh wounds from infection by the oak wilt fungus. Further research suggested augmentation sprays with *Ophiostoma piceae* would be an effective method to block overland spread of oak wilt. In 1993 Dr. French began cooperative research with Dr. Jennifer Juzwik, USDA Forest Service, North Central Forest Experiment Station. USFS, University of Minnesota and a private company funded field research 1993-present.

Other methods of controlling sporulation and overland spread of oak wilt (e.g., red oak removal and disposal or treatment; girdling red oaks to facilitate drying of standing trees) are perhaps 40-60% effective due to timing required, large number of property owners and jurisdictions involved. The Cooperative Suppression Program funded by the USDA Forest Service has pointed out the need for a more effective method of reducing inoculum levels. Many people are willing to pay to control underground spread of oak wilt when the procedure is affordable (there is 50% reimbursement of cost) but in some areas their efforts are threatened by the great amount of inoculum of overland spread in the immediate vicinity of their property. Severe weather during the spring that would wound healthy oaks makes them vulnerable to infection from spores from mats on red oaks in the area.

VIII. Budget context:

For the 2-year period ending June 30, 1995 the USDA Forest Service, North Central Research Station, University of Minnesota and S. Kunde Company, Forestry Consultants spent $23,500, $7,000 and $6,500, respectively, on early trials to evaluate the efficacy of this method.

The Minnesota Department of Agriculture has agreed to match the $10,000 grant from LCMR to perform Objective C. $5,000 will be contributed to the research effort for this objective in each year of the two-year period beginning July 1, 1995.

IX. Dissemination:

Data and results of the cost-benefit analysis will be published as a brochure or bulletin describing operational methodology for application. The intended audience will be county and municipal staff responsible for managing oak wilt in their jurisdiction. The Minnesota Department of Agriculture, Shade Tree Program will include this information in training sessions for Certified Tree Inspectors and community foresters. These sessions are held annually at several sites around the state.

General information about the method would be shared in press releases, PSA's on TV, radio, cable, and articles in newspapers. Booths or displays at county fairs, trade shows, Arbor day and Earth day programs are also effective ways to get general information about the method to property owners who could use it. Public meetings and interviews on TV, radio and cable are further ways to get the information out to people.

The results of the studies will also be published in scientific and trade journals, and formal presentations will be made at professional meetings.

X. Time:

Project as outlined will be completed in two years with one exception. Evaluation of the spring 1997 application will not be completed until August 1997. USDA Forest Service cooperation will ensure that evaluation is completed. Additional monitoring to complete the steps leading to full U.S. EPA registration is to be expected. Some additional work will be required to meet the requirements for registration. This will limit commercial availability of the product until all requirements are met. Project cooperators will seek corporate sponsors to assist with this process, as well as work closely with U.S. EPA staff to facilitate this process.

XI. Cooperation:

Dr. David French, Professor Emeritus, University of Minnesota, Department of Plant Pathology, Saint Paul is the investigator who discovered and performed initial investigation into this approach to control the overland spread of oak wilt. He turned further research into the development of this approach over to Dr. Jennifer Juzwik. He will oversee field research, data collection and preparation of updates and reports on the results of the project.

Dr. Jennifer Juzwik, Research Plant Pathologist, USDA Forest Service, St. Paul is the principal investigator on the project for Objectives A and B, including setting up field studies, collecting data, processing samples in the laboratory, analyzing data and writing up

reports. She will also contribute data and information needed for Objective C. She will dedicate 25% of her time to the project.

Mr. Charlie Evenson, Forestry Specialist, Hennepin County Parks, Plymouth will provide access to field study locations for Objective A and provide application equipment and technical assistance for Objective B research. He will also contribute data and information needed for Objective C. Fifteen percent of his time during summer 1995 and spring 1996 will be required.

Mr. Dan Gillman, Oak Wilt Program Coordinator, MN Department of Agriculture, St. Paul will serve as Program Manager to oversee the project and help prepare update reports to LCMR. He will also be the lead person for Objective C, Conduct cost-benefit analysis comparing biocontrol of overland spread of oak wilt with current approaches used. Twenty-five percent of his time will be required to serve as Program Manager and complete Objective C.

Dr. Pamela Jakes, Ecological Economics Unit, USDA Forest Service, St. Paul will provide step-by-step guidance in conducting environmental and socio-economic benefits and cost analysis of biologically controlling overland spread of oak wilt with *Ophiostoma piceae* in comparison to current approaches. Fifteen percent of her time during winter 1996-97 will be devoted to Objective C.

.II. Reporting Requirements:

Semiannual six-month work program update reports will be submitted not later than January by June 30, 1997. 1,1996, July 1, 1996, January 1, 1997 and a final six-month work program update and final report

XIII. REQUIRED ATTACHMENT:

Qualifications:

Curriculum Vitae of the Project Originator and Research Investigator, Dr. David French, the Principal Investigator, Dr. Jennifer Juzwik and, the Project Manager, Mr. Daniel Gillman, are attached.

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Table 1. Oak wilt mat colonization by *Ophiostoma quercus* in Ham Lake, MN, *O. quercus* spray trials. Number of mats with different frequency of isolation is reported by mat age.

Mat age[1]	Treatment[2]	Mat height category[3]	No. trees yielding sampled mats	Percentage of sub-samples yielding *O. quercus*[4]							Statistical results[5]
				0	17	33	50	67	83	100	
				Number of mats							
younger	*O. quercus*	lower	2	0	0	0	0	2	1	3	a
	O. quercus	upper	3	1	2	0	0	1	0	2	ab
	none	lower	6	3	4	1	1	1	3	1	b
	none	upper	2	4	0	3	2	0	0	0	b
older	*O. quercus*	lower	10	1	2	3	3	4	3	9	c
	O. quercus	upper	9	1	5	4	3	2	1	7	c
	none	lower	7	7	1	2	3	3	4	5	cd
	none	upper	7	12	3	1	0	2	4	3	d

[1] Younger includes mat ages 1, 1.5, 2 and 2.5; older includes mat ages 3, 3.5 and 4. These specific categories are based on categories described by Curl (1955).

[2] Either *O. quercus* spray or none (no spray).

[3] Lower includes mats collected from the main stem at heights of < 4 m from the ground. Upper includes mats collected from the main stem at heights between 4 and 9 m.

[4] Data were derived from isolation of *O. quercus* from six 3-mm-diameter mat subsamples using serial dilution plating techniques.

[5] Results of singly ordered Kruskal-Wallis analyses comparing distributions across mat age. Distributions followed by different letters were not similar ($P < 0.05$).

Table 2. Oak wilt mat colonization by *Ophiostoma quercus* in Eagan, MN, *O. quercus* spray trial. Number of mats with different frequency of isolation is reported by mat age.

Mat age[1]	Treatment[2]	Mat height category[3]	No. trees yielding sampled mats	Percentage of sub-samples yielding *O. quercus*[4]							Statistical results[5]
				0	17	33	50	67	83	100	
				Number of mats							
younger	O. quercus	lower	6	0	0	1	0	3	8	12	a
	O. quercus	upper	8	1	2	2	0	4	3	11	a
	none	lower	6	10	5	2	1	2	1	2	b
	none	upper	3	18	4	3	1	1	1	1	b
older	O. quercus	lower	11	0	0	0	2	2	8	11	a
	O. quercus	upper	8	0	0	1	3	3	10	15	a
	none	lower	5	5	5	0	0	2	2	9	c
	none	upper	5	2	0	2	2	1	1	16	a

[1] Younger includes mat ages 1.5, 2,2.5, and 3; older includes mat ages 3.5 and 4. These specific categories are based on categories described by Curl (1955).

[2] Either *O. quercus* spray or none (no spray).

[3] Lower includes mats collected from the main stem at heights of < 4 m from the ground. Upper includes mats collected from the main stem at heights between 4 and 9 m.

[4] Data were derived from isolation of *O. quercus* from six 3-mm-diameter mat subsamples using serial dilution plating techniques.

[5] Results of singly ordered Kruskal-Wallis analyses comparing distributions across mat age. Distributions followed by different letters were not similar ($P < 0.05$).

Table 3. Number of colony-forming units (CFU) of *Ophiostoma quercus* isolated from oak wilt mats in Ham Lake and Eagan, MN, *O. quercus* spray trials.

Location	Mat Age Category[1]	Treatment[2]	Mat collection height[3]	Mats yielding *O. quercus*[4]	
				Total	Average CFU/cm^2 (x10^3)
Ham Lake	younger	*O. quercus*	low + high	11	34 b[5]
		none	low + high	16	5 a
	older	*O. quercus*	low + high	51	220 c
		none	low + high	30	270 c
Eagan	younger	*O. quercus*	low + high	46	420 e
		none	low + high	24	15 d
	older	*O. quercus*	low	23	790 g
			high	32	1200 i
		none	low	18	750 f
			high	22	850 h

[1] Age based on descriptions by Curl (1955). For Ham Lake mats, younger category includes mat ages 1.5, 2 and 2.5; older includes mat ages 3, 3.5, and 4. For Eagan mats, younger category includes 1.5, 2, 2.5 and 3; older includes mat ages 3.5 and 4.

[2] Either *O. quercus* spray or no spray (none).

[3] Mats collected < 4 m = "low" while mats collected between 4 and 9 m = "high". Categories combined when no statistical differences between low and high mats were found.

[4] Data were derived from isolation of *Q. quercus* from six 3-mm-diameter mat subsamples using serial dilution plating techniques.

[5] Values followed by different letters within the column are not similar ($P < 0.05$) based on ANOVA.

Table 4.—Summary of responses to questionnaire completed by managers of oak wilt control programs in Minnesota communities

Question	Response		
	Average	Median	Range
Q3.—How long has your community had an oak wilt control program?	11 years	10 years	3-22 years
Q6.—Over the course of a year, approximately what percentage of your time is spent on oak wilt control?	12 percent	5 percent	1-66 percent
Q16.—About how much per year does your community spend on oak wilt control?	$12,300	$7,000	$200-$39,000
Q17.—Approximately what percent of Q16 are spent on private lands	74 percent	90 percent	0-100 percent
Q18.—Approximately what percent of Q16 are covered by outside sources?	37 percent	34 percent	0-100 percent
Q19.—What is the average cost of tree removal in your area?	$343	$300	$100-$600
Q20.—What is the average cost of breaking root graphs around an infection center?	$439	$475	$100-$750
Q22.—What percent of the costs of oak wilt control on private property is covered by the private landowner?	67 percent	50 percent	5-100 percent
Q23.—What percent of the time do you get complete compliance with your treatment recommendation on private land?	71 percent	85 percent	5-100 percent