

Special Technology Development Program Final Report

PROJECT NUMBER NA-1999-5

PROJECT TITLE Development of a Molecular Protocol for Detecting the Ash Dieback Bacterium, a New Threat to Northeastern Forests

PROJECT STATUS:

Completed (work ended this fiscal year)

EXPECTED PROJECT DURATION: 2

EXPECTED COMPLETION DATE OF THE PROJECT (fiscal year): FY 2000

ACTUAL COMPLETION (FISCAL) YEAR: FY 2001

SUBJECT: Disease Management: Survey, Evaluation and Monitoring; pathogen detection; ash dieback bacterium

STATUS OF SUBJECT SPECIES: native

PROJECT OBJECTIVE(S): To develop an operational protocol for sample collection, DNA extraction and specific detection of the ash dieback bacterium (ADB).

BRIEF DESCRIPTION OF PROJECT: A newly-discovered disease is causing extensive damage to Iowa's ash trees. The distribution and extent of the disease is unknown. Symptoms are easily confused with the less serious ash yellows disease. Existing methods (e.g. DAPI tests) for detection cannot distinguish between the ash yellows phytoplasma and the new bacterium. This project is to develop a new laboratory protocol (based on PCR technology) to detect the putative bacterial agent and distinguish it from the ash yellows phytoplasma. The project focuses on developing a reliable and efficient sampling method with a highly specific set of PCR primers.

CHANGES TO PROJECT SCOPE OR OBJECTIVES (Changes that need to be made to the original proposal and reasons for the changes.):

ADDITIONS TO PROJECT SCOPE OR OBJECTIVES (Describe additional accomplishments expected from the project.):

FHP LEAD CONTACT (FHP person submitting proposal):

<u>Name</u>	<u>Affiliation (Office or Dept.)</u>	<u>Phone, E-mail, Fax</u>
Linda Haugen		

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

<u>Name</u>	<u>Affiliation (Office or Dept.)</u>	<u>Phone, E-mail, Fax</u>
Tom Harrington,	Professor of Plant Pathology, Iowa State University (ISU)	(515) 294-0582 tcharrin@iastate.edu FAX: (515) 294-9420

COOPERATORS (contributing to, but not leading, the project) (add lines as necessary):

<u>Name</u>	<u>Affiliation (Office or Dept.)</u>	<u>Phone, E-mail, Fax</u>
Chris Feeley	Department of Forestry, ISU	(515) 294-6739 cfeeley@iastate.edu
Woody Hart	Dept. of Entomology, ISU	(515) 294-8623 ehart@iastate.edu

COOPERATOR INVOLVEMENT (add lines as necessary):

<u>Name</u>	<u>Role</u>	<u>Time Commitment</u>
Tom Harrington, Professor of Plant Pathology, Iowa State University (ISU).	Coordination of the project, experimental design, assessment of results, writing of manuscripts.	(5% of time).
Chris Feeley, Graduate Research Assistant, Forestry, ISU.	Location of sample trees, sample collection, training of hourly employees.	(25% of time).
Woody Hart, Professor of Entomology, ISU.	Co-advisor of Chris Feeley.	(less than 5% of time).

PRODUCTS AND DUE DATES: A standard protocol for type of sample, DNA extraction method, and PCR analysis will be developed for detection of the ash dieback bacterium. The sampling protocol will include the suitable times of the year and the type of plant tissue (midribs, petioles, or inner bark of twigs, branches, stems or root flares) to be collected. This procedure will be published in a refereed journal and will be freely available to the public. Publication will be in the year 2000, though we will make the procedure available in late 1999.

STATUS OF PRODUCTS/PRESENTATIONS: Although we were not successful in developing the diagnostic procedures for the new bacterium, we were able to use the procedure for detecting the ash yellows phytoplasma. This work was included in Chris Feeley's M.S. Thesis and in a publication in the Journal of Arboriculture. Also, a Forestry Extension Note was presented to help understand the green ash decline and diagnose the disease based on symptomatology.

ACCOMPLISHMENTS TO DATE:

Publications:

Feeley, C. J. 2000. Ash Decline in Iowa. M.S. Thesis. Iowa State University. 64 pp.

Feeley, C.J., E.R. Hart, J.R. Thompson, and T.C. Harrington. 2001. Occurrence, associated symptoms, and potential insect vectors of the ash yellows phytoplasma in Iowa. J. Arb. 27(6): 331-339.

Technology Transfer:

Feeley, C. J. 2001. Understanding ash decline. ISU Forestry Extension Note F-373. 2 p.

BRIEF DESCRIPTION OF ACCOMPLISHMENTS AND RESULTS: We did develop a protocol for sampling symptomatic ash trees, extracting DNA and performing PCR tests. However, we were not successful in developing the specific primers for the bacterium that we think is responsible for the decline of green ash in Iowa. We found that the bacterium identified in preliminary work is not consistently associated with the disease and therefore could not prove that it was the causal agent. We used the same protocol for detecting the ash yellows phytoplasma, and we found that it, too, is not consistently associated with the disease. The protocol is useful for diagnosis of phytoplasma caused diseases of trees, and it will also be useful if or when primers are available for the putative agent of the green ash problem.

DOCUMENTATION: The Journal of Arboriculture article is readily available in most technical libraries, and reprints are available on request. The ISU Department of Forestry has the Extension Note F-373 available free of charge upon request.

FIRST FISCAL YEAR FUNDED:

FUNDS OBLIGATED FROM BEGINNING OF PROJECT THROUGH FINAL FISCAL YEAR (extend table as needed):

Fiscal Year	STDP Funding	Other-Source funding	Source
1999	18,000	4000	Iowa Landscape and Nurseryman's Association
		9000	Iowa State University-Salary
		6000	Iowa State University-Special Research Initiation Grant
2000	4,000	4000	Iowa State University-Salary

FUNDS NOT USED FROM PREVIOUS FISCAL YEAR:

If there are unused funds, what is the reason for not using them?

POST-PROJECT TECHNOLOGY SUPPORT:

We have no plans to pursue this project further or for working on this disease. The research effort would require substantially more funding than has been available.

LOOK TO THE FUTURE:

As explained above, we have had more difficulties than expected in primer development. This has proven to be a difficult disease to work on, as have similar diseases in a number of other crops. However, the work to date strongly supports our initial contention that the causal agent is not the ash yellows phytoplasma. The problem remains important, but elucidation of the etiology of the disease will require a more substantial research effort with considerably more funding than has been available.