

FHP SPECIAL TECHNOLOGY DEVELOPMENT PROJECT PROGRESS REPORT

PROJECT STATUS: Continuing

PROJECT NUMBER: NA-1999-5

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

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

PROJECT OBJECTIVE: 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.

FHP PERSON WHO WILL LEAD THE PROJECT: Linda Haugen

COOPERATORS:

Linda Haugen, U.S. Forest Service Forest Health Protection.

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).

BRIEF DESCRIPTION OF ACCOMPLISHMENTS AND RESULTS: We sampled symptomatic white and green ash trees monthly from April 1999 through October 1999. Leaf samples and twig samples were taken from 10 selected trees in Ames and six selected trees in St. Paul. Extractions were made from mid-ribs and the inner bark (phloem) of twigs. Inner bark samples have proven to be as effective as mid-rib samples, and the inner bark samples are easier to prepare. We have tried a number of different extraction techniques, and the most consistent results were obtained with the most laborious technique, a phenol-chloroform extraction.

These DNA extractions will be used for template for the polymerase chain reaction (PCR) to see if the ash dieback bacterium and/or the ash yellows phytoplasma is present in the symptomatic but not diseased trees, to see if leaf or twig samples are more effective, and to see at which times of the year the pathogen can be detected.

We have had difficulty in developing primers that are specific to the ash dieback bacterium. The primers utilized in 1998 generally give a PCR product with DNA extracted from symptomatic trees, but some apparently non-symptomatic trees also prove positive with these primers. We believe that there are numerous secondary bacteria in the symptomatic trees, and the primers we designed may be amplifying rDNA in a secondary bacterium rather than the primary causal agent. We have designed numerous other primers for other bacteria isolated from symptomatic trees, but none have proven successful in differentiating symptomatic from non-symptomatic trees. Once better primers are developed, we will use the extracted DNA samples to test for the presence of the ash dieback bacterium and the ash yellows phytoplasma.

We have developed an effective PCR protocol for detecting the ash yellows phytoplasma, but thus far we have only detected the phytoplasma with DNA extracted from witches' brooms of naturally-infected trees. We are currently testing the protocol with graph-inoculated green and white ash cultivars graft-inoculated with the ash yellows phytoplasma. This testing of the protocol will soon be completed.

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

FIRST YEAR FUNDED: FY 1999

YEAR SCHEDULED TO END: FY 2001

ACTUAL YEAR TO END: FY 2001

PRODUCTS: 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. Two presentations have been made on the new disease: at a FHP cooperators meeting in St. Paul and a vascular wilt workshop conducted by the American Phytopathological Society.

STATUS OF PRODUCTS: No refereed publications at this date, but one M.S. thesis completed.

FUNDS OBLIGATED FROM BEGINNING OF PROJECT THROUGH END OF FY 2000:

Fiscal Year	STDP funding	Contributions	Source organization
2000	4,000	4000	Iowa State University-Salary

FUNDS CARRIED OVER FROM FY200 to FY2001: These funds will be spent by 31 March 2001.

POST-PROJECT TECHNOLOGY SUPPORT: None anticipated at this time.

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. Our initial confidence in the identity of a bacterial causal agent has been proven shaky, and we will continue to explore the possibilities of other causal agents. However, the work to date strongly supports our initial contention that the causal agent is not the ash yellows phytoplasma.