Searching for the Oak Wilt Pathogen, *Ceratocystis fagacearum*, in New York State

Emma Rosenthal, Karen Snover-Clift, Sandra Jensen

Cornell University, Ithaca, NY

Plant Disease Diagnostic Clinic

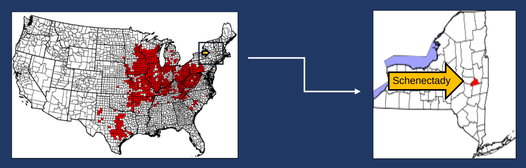
Cornell University

NEW YORK STATE OF OPPORTUNITY Agriculture and Markets

NEW YORK STATE OF OPPORTUNITY Department of Environmental Conservation

History

Oak wilt is a disease specific to oak trees, particularly red oaks, that is caused by the fungal pathogen *Ceratocystis fagacearum*. First identified in the US in 1942, this disease has spread across the Midwest and Mid-Atlantic and caused considerable damage. In 2008, the first case of the disease was identified in New York State in Schenectady county. After a substantial eradication effort made by the New York State Department of Environmental Conservation (NYSDEC) and the New York State Department of Agriculture and Markets (NYSDAM), the disease again appeared in 2013 in the same neighborhood. Again, efforts were made to control the pathogen. Because it was identified twice in upstate NY, members of the Plant Disease Diagnostic Clinic proposed a broader investigation to determine whether the oak wilt infection in Schenectady county was a unique situation that had been contained or if the pathogen is currently present elsewhere in the state, which would necessitate further eradication effort.



Facts About Oak Wilt Infection

- All species of oak are susceptible to oak wilt to some degree, but Red oaks are far more susceptible than White oaks. Reds may die within weeks, while Whites may die over several years.
- The major signs and symptoms include:
  - Wilting in June or July
  - Marginal "scorch" on leaves is sometimes present
  - Vascular discoloration is sometimes present
  - Fungal pads on the inner bark that smell of rotting fruit
  - Cracks in the bark if the fungal pads produce enough pressure

William M. Ciesla, Forest Health Management International, Bugwood.org

C.E. Skelisar, Bugwood.org

Sandra Jensen, Cornell University, Bugwood.org

USDA Forest Service – Forest Health Protection, Bugwood.org

Our Project

In 2015, The Plant Disease Diagnostic Clinic at Cornell University partnered with the NYSDEC and the NYSDAM to survey the state for evidence of oak wilt. A new sample submission form was developed, as well as informational cards, posters, and an article that were distributed around the state. Our goal was to spread the word about the potential for oak wilt to be spreading into the state and to warn trained arborists and the public alike to be on the look out.

Background Photo: Paul A. Mistretta, USDA Forest Service, Bugwood.org

Spreading awareness about the potential presence of oak wilt had the effect we were hoping – suspect samples were mailed to the Clinic by concerned arborists. Additionally, several sample collection trips were made by the Clinic alongside members of the NYSDEC.

Totals
- Samples Received: 30
- Samples Processed: 25
- Samples Pending: 5

In 2015
- Samples Received: 22
- Samples Processed: 22
- Samples Pending: 0

In 2016
- Samples Received: 8
- Samples Processed: 3
- Samples Pending: 5

A major aspect of the project was to determine whether our oak wilt diagnostic protocol could be effective by testing tissue directly, instead of only using fungal cultures as had been the practice during previous investigations. Being able to test branch tissue directly would speed up the procedure significantly by cutting out the time it takes to grow the pathogen in culture, which is typically 10 days.


Suspect sample received by the Clinic in 2016; bark is peeled off to reveal vascular tissue.

This project is made possible by the Specialty Crop Block Grant administered by the New York State Department of Agriculture and Markets.

Methods

1. Sampling
- Samples gathered by a Clinic technician and members of the NYSDEC during surveys around NYS.
- Samples submitted by the Cornell Cooperative Extension and trained arborists.
- Each sample included branch or bark tissue from suspect trees.

2. Generating Sub-samples
- Every sample received was split into 2 sub-samples:
  - One: 180-200 mg of branch or bark tissue was cut and put into a 1.5 mL tube for DNA extraction.
  - Two: small sections of branch tissue were cut and plated onto Acidified Potato Dextrose Agar to encourage fungal growth.

3. DNA Extraction
- DNA extraction was performed on the branch tissue using QIAamp® Fast DNA Stool Mini Kit.
- Isolated fungal specimen was collected in a 1.5 mL tube and DNA extraction was performed using the Qiagen DNeasy® Plant Mini Kit.

4. Conventional PCR
- Both sub-samples were run through a nested PCR, designed with specificity to amplify DNA associated with *Ceratocystis fagacearum*.
- Diluted versions (1:10) of every sub-sample were also run through the same reactions.
- Positive control was DNA extract known to be *Ceratocystis fagacearum*; negative control was molecular grade water.

5. Gel Electrophoresis
- All PCR products from suspect samples, diluted samples, and controls are run through a 2% agarose gel at 100V.
- A band at 280 bp is expected for a positive sample of *Ceratocystis fagacearum*.
- A 100 bp ladder is used as a reference.

6. Purification & Sequencing
- Any PCR product that produced a banding pattern during gel electrophoresis was then purified and sent away for sequencing.
- Sequencing was performed by the Cornell Biotechnology Resource Center.
- The National Center for Biotechnology Information's Basic Local Alignment Search tool was used to analyze sequence data.

Results

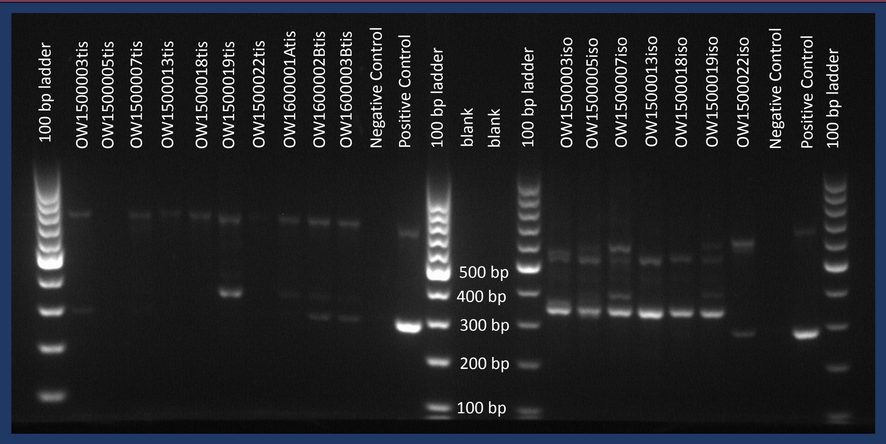


Figure 1. 2% Agarose gel electrophoresis using PCR products from nested conventional PCR procedure. This gel run is a third or fourth replicate for each sample. Included are only samples which presented suspect results in previous runs.

- The nested conventional PCR procedure that was used here, first published in Wu et al. (2011), was developed to be specific to *C. fagacearum* by analyzing similarities between 128 sequences of *Ceratocystis* spp. from GenBank and designing primers that would perform with the highest specificity to the pathogen.
- Positive controls confirm the expected 280 bp band for *C. fagacearum*.
- Several samples displayed faint banding at 280 bp; these were later sequenced.
- A number of the samples show a band at 370 bp; these were also sequenced.

Sample Number	Sequencing Result	% ID
OW1500003iso	*Cladosporium sp.*	99%
OW1500005iso	*Cladosporium sp.*	98%
OW1500007iso	*Cladosporium sp.*	98%
OW1500013iso	*Cladosporium sp.*	100%
OW1500018iso	*Cladosporium sp.*	99%
OW1500019tis	*Cladosporium sp.*	99%
OW1500022iso	*Ceratocystis fagacearum*	99%
OW1600001tis	*Ceratocystis fagacearum*	99%
OW1600002tis	*Ceratocystis fagacearum*	95%
OW1600003tis	*Ceratocystis fagacearum*	98%
Positive Control	*Ceratocystis fagacearum*	100%

Table 1. Results of sequencing purified DNA from suspect samples. Two to four replicates were completed for each sample; this data was generated using NCBI's Basic Local Alignment Search Tool (BLAST).

- Samples that before exhibited an unexpected 370 bp band, in either branch or culture sub-sample, have greatest similarity to a fungus of the genus *Cladosporium.*
- All samples exhibiting a 280 bp band have great similarity to a fungal isolate of *C. fagacearum*.
- One sample that did not here exhibit a band at 280 bp has great similarity to a fungal isolate of *C. fagacearum*.

Conclusions & Future Research

1) The diagnostic procedure designed to be specific to *C. fagacearum*, is also capable of amplifying DNA belonging to fungi of the genus *Cladosporium*, a common secondary invader of decaying tissue and likely not a cause of disease.
2) We have yet to isolate the oak wilt pathogen in culture. Our suspect samples arrived late in the season, and experts say that isolating the pathogen in the late fall and winter is difficult. We will attempt to culture again in the Spring.
3) One of our molecular tests suggests (up to 99% certainty) that we have identified *C. fagacearum*, and we are developing a second PCR method with the hopes of gaining 100% certainty through our new testing procedure.