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 (NYSADM), 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 efforts.

**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

**Our Project**

In 2015, The Plant Disease Diagnostic Clinic at Cornell University partnered with the NYSDEC and the NYSADM 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 on the look out.

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.

**Methods**

1. **Sampling**
   - 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.

2. **Generating Sub-samples**
   - All PCR products from suspect samples, diluted samples, and controls are run through a 2% agarose gel at 100V.
   - A band at 300 bp is expected for a positive sample of *Ceratocystis fagacearum*.
   - A 100 bp ladder is used as a reference.

3. **DNA Extraction**
   - Both sub-samples were run through a nested PCR, designed with specificity to amplify DNA associated with *Ceratocystis fagacearum*.
   - Diluted versions (1/5 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.

4. **Conventional PCR**
   - The nested conventional PCR product 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.

5. **Gel Electrophoresis**
   - A band of 280 bp was expected for a positive sample of *Ceratocystis fagacearum*.
   - A 100 bp ladder is used as a reference.

6. **Purification & 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**

- **Sample Number Sequencing Result % ID**
  - OW1500002iso *Ceratocystis fagacearum* 99%
  - OW1500019tis *Cladosporium sp.* 98%
  - OW1600001tis *Ceratocystis fagacearum* 99%
  - OW1600002tis *Ceratocystis fagacearum* 98%
  - OW1500007iso *Cladosporium sp.* 98%
  - OW1500018tis *Cladosporium sp.* 99%
  - OW1500005tis *Cladosporium sp.* 100%
  - OW1500019iso *Cladosporium sp.* 98%
  - OW1500022iso *Cladosporium sp.* 98%
  - OW1600002Btis *Cladosporium sp.* 98%
  - OW1500013iso *Cladosporium sp.* 98%

**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.