Sudden Oak Death and Phytophthora ramorum

New Identification Technologies, Management Strategies and What this Means to You

Karen Snover-Clift
Director, Plant Disease Diagnostic Clinic
Associate Director, NEPDN
National Quality Manager, NPDN
Cornell University

NYSTLA 2015 Professional Turf & Landscape Conference & Trade Show
Yonkers, NY
January 14, 2015

NPDN National Plant Diagnostic Network

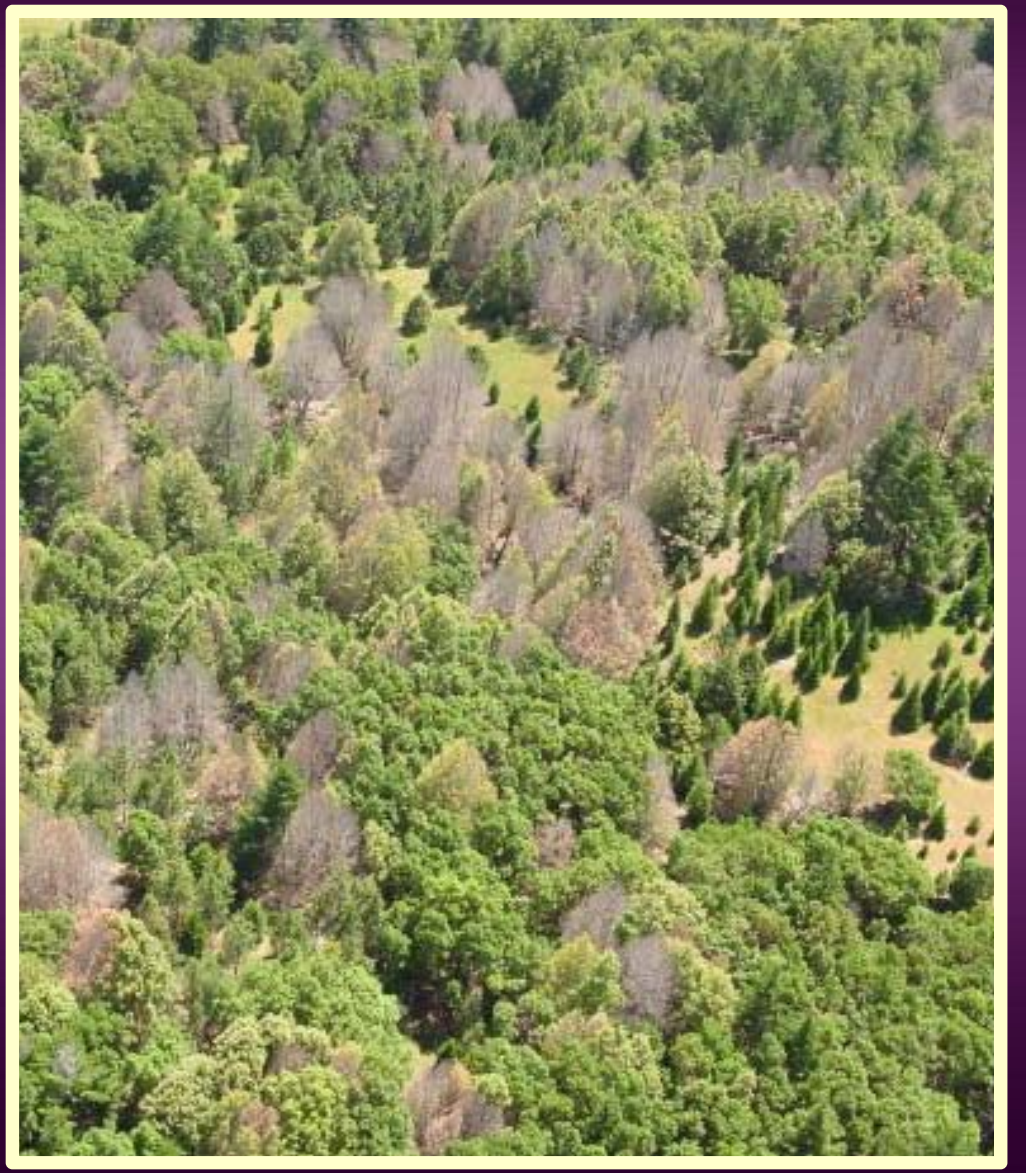
NIFA

NEPDN Northeast Plant Diagnostic Network

Sudden Oak Death and Ramorum Blight, caused by *Phytophthora ramorum*

Sudden Oak Death, *Phytophthora ramorum*

- Also called ramorum blight and ramorum dieback
- Although the disease was first observed in the United States in tanoaks in California, it is also found to infect many other plant species
- Caused a massive trace forward and trace back surveys in 2004 when it was found in a large production nursery in southern California



History of *Phytophthora ramorum*

- **2004** — *P. ramorum* found in Los Angeles, CA nursery
- **2002** — *P. ramorum* isolates from Europe and U.S. determined to differ
- SOD epidemic has spread to 12 CA counties and parts of Curry County, OR
- **2000** — UC Berkeley identifies *P. ramorum* as the cause of SOD
- **1995** — Numerous tanoaks observed dying in Marin County, CA
- **1993** — *P. ramorum* discovered in Germany and the Netherlands

(Year)

(Govt of British Columbia, Ministry of Ag, Food, & Fisheries)

History of *Phytophthora ramorum*

2007-2014 National surveys completed, Observational surveys and trace forwards continue, US Forestry focusing on stream baiting project.

2006 Nothing found in landscape so far. Surveys still focusing on containerized plant material.

2005 Positive findings- 1-South Carolina, 2 Louisiana, 4-Georgia, and 1-Tennessee.

2004 November-Federal Order requires WA, OR, and CA nurseries to test host plants prior to out of state shipment

(Year)

(Govt of British Columbia, Ministry of Ag, Food, & Fisheries)

Common Regulated Hosts

Common Name	Scientific Name
Japanese camellia	*Camellia japonica*
Sasanqua camellia	*Camellia sasanqua*
Witch Hazel	*Hamamelis virginiana*
Himalaya pieris	*Pieris formosa*
Pieris ‘Forest Flame’	*Pieris formosa x japonica*
Pieris ‘Brouwer’s Beauty’	*Pieris floribunda x japonica*
Japanese pieris	*Pieris japonica*
Douglas-fir	*Pseudotsuga menziesii* var. *menziesii*
Coast Live Oak	*Quercus agrifolia*
Rhododendron (including azalea)	*Rhododendron spp*
Bodnant Viburnum	*Viburnum x bodnantense*
Doublefile Viburnum	*Viburnum plicatum* var. *tomentosum*

Common Plants associated with *P. ramorum*

Common Name	Scientific Name
Grand fir	*Abies grandis*
Horse-chestnut	*Aesculus hippocastanum*
Camellia	*Camellia reticulata*
Camellia	*Camellia* x *williamsii*
European beech	*Fagus sylvatica*
Mountain laurel	*Kalmia latifolia*
Drooping leucothoe	*Leucothoe fontanesiana*
Chinese pieris	*Pieris formosa* var. *forrestii*
Pieris	*Pieris formosa* var. *forrestii* x *Pieris japonica*
Formosa firethorn	*Pyracantha koidzumii*
Southern red oak	*Quercus falcata*
Northern red oak	*Quercus rubra*
Salmonberry	*Rubus spectabilis*
Lilac	*Syringa vulgaris*
David Viburnum	*Viburnum davidii*
Fragrant Viburnum	*Viburnum farreri*
Wayfaringtree Viburnum	*Viburnum lantana*
Burkwood Viburnum	*Viburnum x burkwoodii*
Prague Viburnum	*Viburnum x pragense*

Sudden Oak Death Symptoms

Bleeding Bark Canker

Twig Dieback

Foliar Blight

(Photos Joseph O'Brien, USDA Forest Service, www.forestryimages.org)

2004-Tiffany Creek Preserve

Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

2004-Tiffany Creek Preserve

Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

Types of Sampling and Testing

Visual Surveying: Inspectors visually surveyed areas for characteristic symptoms of a *P. ramorum* infection. Symptomatic leaf or shoot lesions were selected and collected for analysis.

Water Filtration: Water collected from retention ponds was filtered through polycarbonate filtration membranes using a vacuum filtration system. The membranes were then incubated on agar plates for three days in the dark prior to examination. The mycelium of characteristic *Phytophthora*-like growth was harvested and retained for ELISA and PCR processing.

Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

Types of Sampling and Testing

Soil Baiting: Soil samples were placed in aluminum pie pans with distilled water and clean bait leaves (rhododendron) were floated on top for three days. The leaves were collected, incubated and symptomatic tissue was retained for additional testing by ELISA and PCR.

Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

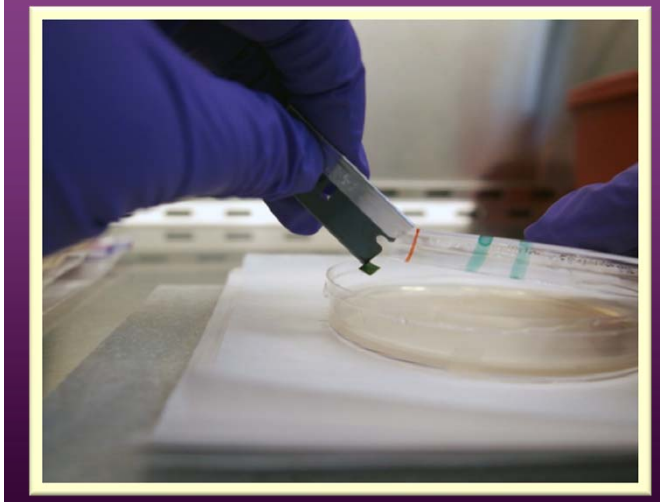
Types of Sampling and Testing

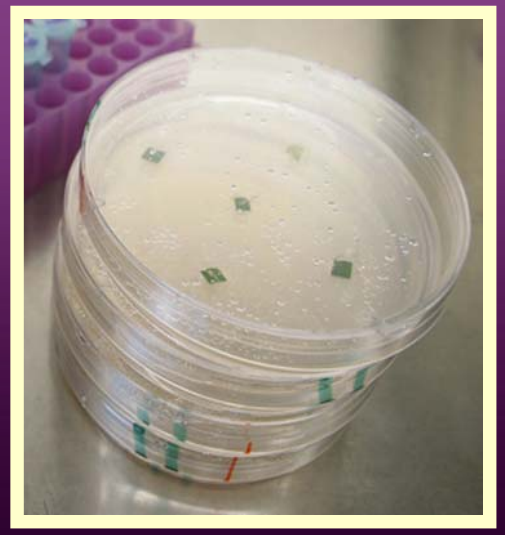
Pond or Stream Baiting: Mesh bags were constructed and bait leaves inserted. The bags were floated in randomly sampled and/or suspect water bodies for seven days during periods when the water temperature was less than 24°C. The leaves were collected, incubated and symptomatic tissue was retained for additional testing by ELISA and PCR.

Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

Types of Sampling and Testing

Bottle of Bait (BOB): This technique was used at sites where mesh bags used for pond or stream baiting could not float on top of the water, such as in small puddles of irrigation run-off. One intact, non-wounded rhododendron leaf and 20 rhododendron leaf pieces (~4mm circle/triangle/square) were added to a bottle of water. The bottle was positioned on its side (so that the intact leaf and pieces floated on the water surface) and incubated for 3 days.



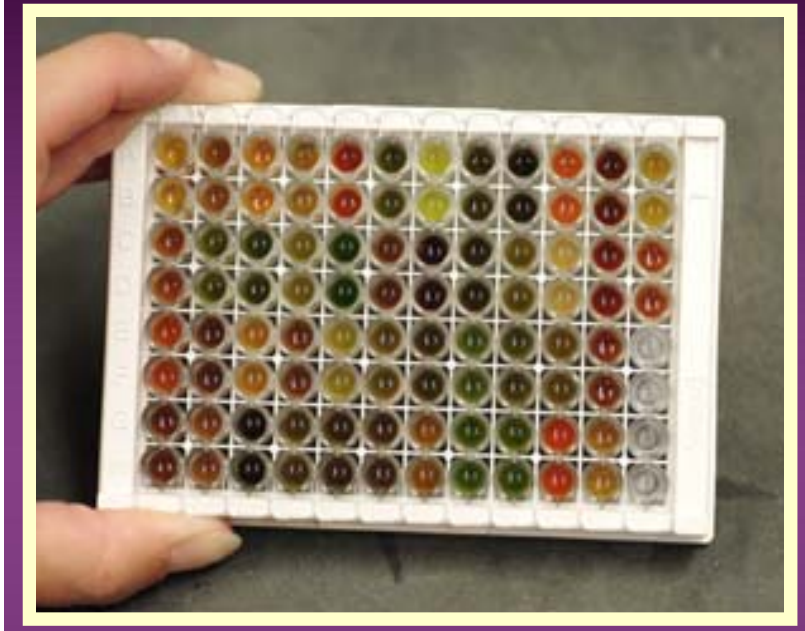


Culturing

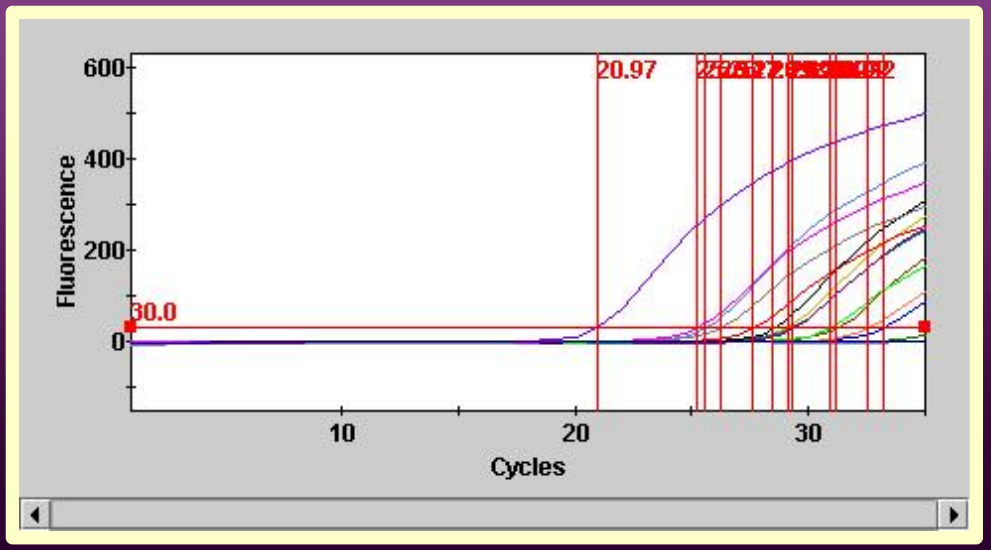
Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

Types of Sampling and Testing

ELISA



PCR



Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

Early Surveying for New York State, 2004-2009

- Conducted testing for NYSDAM, USFS-some Northeast states, and Trace Forwards for USDA-APHIS.

- 2004-1517 samples
- 2005-676 samples
- 2006-442 samples
- 2007-62 samples
- 2008-58 samples
- 2009-17 samples

- All samples processed in our laboratory tested negative for *Phytophthora ramorum* for the six years of 2004-2009.

Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

First positive find in NYS-2010

- In 2010, the samples were handled a bit differently because of Farm Bill funding with NYSDAM, samples sent directly to the USDA-APHIS-PPQ Regional Laboratory for testing.
- A Long Island nursery retention pond sample produced a positive result, our lab asked to do some follow-up analysis.
- November 10th, we received 12 soil samples and 4 water samples.

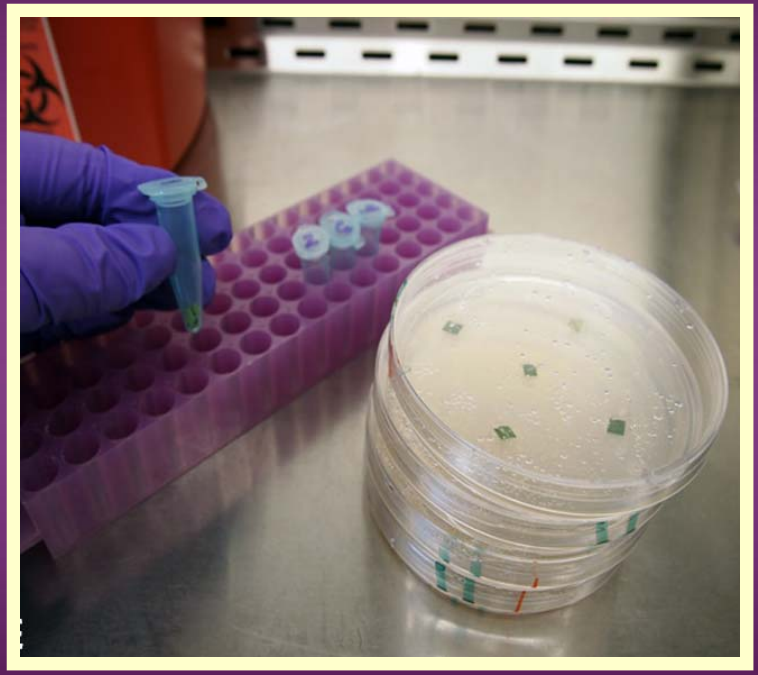
Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

Retention Ponds

Retention Ponds

First positive find in NYS-2010

- Samples were processed using soil baiting and water filtration techniques.
- From the 16 samples, we tested 139 subsamples from cultures using ELISA and positive ELISA samples were tested using real time PCR.
- All of the 139 samples produced negative results.



Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

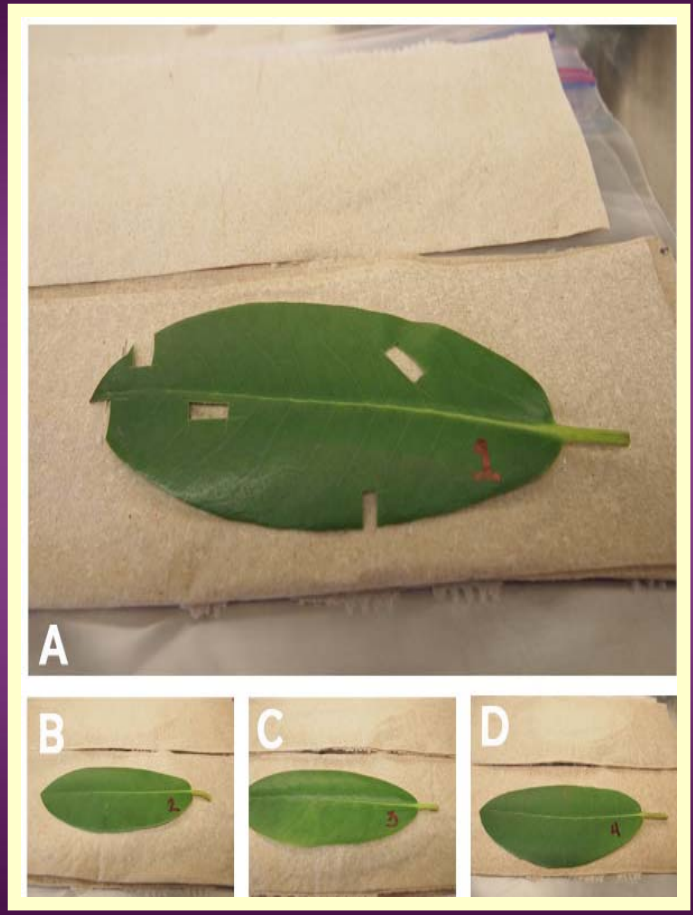
First positive find in NYS-2010

- To continue analysis, on-site water baiting was performed and 4 samples submitted.
- We tested the samples directly with real time PCR.
- One sample produced a strong positive result using ITS and Elicitin PCR, one sample was slight over the threshold levels (FAM Ct=36.53), and the other 2 samples were negative. Samples were submitted to the Beltsville Laboratory for confirmation.

Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

NYS Sampling-2011

- In 2011, 25 samples were submitted from NYSDAM (11), USFS (10) and NEPDN (4) members and tested using the appropriate test method.
- All samples produced negative results, including those submitted from the retention pond that produced positive results in 2010.



Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

NYS Sampling-2012

- **Additional positive site found in 2012!**
- Sampling in 2012 started on April 10th.
- A large batch of samples was submitted on April 24th, 3 samples produced positive results from the previously positive retention pond.
- A second site also produced 3 positive results.
- All samples were submitted to the Beltsville Laboratory for confirmation.

Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

NYS Sampling-2012

- 05-18, 8 soil samples, 2+, site 1.
- 06-21, 23 plant samples, 11+, site 2.
- 07-24, 9 plant samples, 5+, site 2.
- 07-27, 17 plant samples, 2+, site 1.

- Many other sets submitted in between these dates and afterwards.

- Received a total of 215 samples, 139 of those NYSDAM with 26 total positives for the year…
- 6 retention pond (sites 1 & 2),
- 2 soil samples (site 1),
- 18 plant samples (7 site 1 & 11 site 2).

Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

NYS Sampling-2013

- 06-20, 4 plant samples, 2+, site 1.
- 06-27, 5 plant samples, 4+, site 1.
- Two were additional leaves from the first two samples and one was another 'Nova Zembla' in the block and one was a plant that caught their eye while collecting.
- 07-11, 7 plant samples, 3+, site 1.
- Cultures of 5 plant submissions attempted at two locations. Only one produced characteristic growth, it tested positive using qPCR.
- Many other sets submitted before, in between and after these dates.
- Received a total of 252 samples, 127 of those NYSDAM with 10 total positives for the year…
- 9 newly introduced, containerized plant samples and 1 culture (site 1).

Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

NYS Sampling-2014

- **Additional positive site found in 2014!**
- 05-22, 3 leaf baits, 3+, site 2.
- 05-28, 3 leaf baits, 3+, site 3.
- 05-28, 12 leaf baits (4 retention ponds), 0+, site 1.
- 06-18, 6 plant samples, 2+, site 2.
- 07-22, 2 BOB samples, 1+, site 2.

- Many other sets submitted before, in between and after these dates.
- Received a total of 385 samples (150) so far, 9 of those positives…
- 6 leaf baits, 2 newly introduced, containerized plant samples and 1 BOB. Positives from sites 2 and 3.

Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

Sampling Summary through 2014

Beginning in 2004 and through the end of the 2014 survey submissions, which covers 11 years of sample processing, we received a total of **3,441 samples, 47 of those produced positive results**…

Site 1= first confirmed identification in 2010.
Since then 8 leaf bait +, 2 soil +, 16 plant +, and 1 culture +.

Site 2= first confirmed identification in 2012.
Since then 3 leaf bait +, 13 plant +, and 1 BOB.

Site 3= first confirmed identification in 2014.
3 leaf bait +.

Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

What we learned so far…

- Our ability to detect *P. ramorum* has improved through our and others experience with sampling and testing methodology…we detected the pathogen in run off water, soil and containerized plants

1. recommend our inspectors do not collect during hot weather, if look at our testing results, no positives during July and August;
2. no longer test water, thought if received within 24 hours OK but learned from APHIS personnel (Don Seaver) that must arrive within 8 hours, not possible from LI so no longer process water.
3. waste of time to test asymptomatic plants.

- Feel early detection possible with water baiting and BOB. Can cover large nurseries by baiting retention ponds and puddles. Hopeful early detections allow outbreaks to be eradicated before establishment can occur.

Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

Specialty Crops Block Grant

Three objectives to this project…

1. Process all NY samples with ELISA and Immunostrip to determine the consistency we see with our NY samples,

2. Perform *Phytophthora kernoviae* ITS1 and ITS2 qPCR testing on all NY samples submitted this year,

3. and sequence all the ELISA *Phytophthora* positive-*P. ramorum* negative samples to determine which species are commonly found in New York State.

Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

Phytophthora kernoviae

Phytophthora kernoviae

- There are no reports of *Phytophthora kernoviae* in the United States and as far as we know, there are no active survey efforts
- It was first identified in 2003 on a European beech by a scientist from the United Kingdom Forestry Commission and at the same time on a rhododendron by another scientist from the Central Science Laboratory
- The identifier named this new Phytophthora after the city where the infected European Beech was located, it comes from the Cornish name for Cornwall =Kernow


United Kingdom Forestry Commission

Phytophthora kernoviae

- This pathogen is thought to be much more virulent than *Phytophthora ramorum* and therefore is of great concern
- The Europeans are actively looking for this pathogens as part of their surveys for *P. ramorum*
- They are thinking it is an exotic pathogen of recent introduction but the origin is not known
- It has been documented in Europe, Wales and Scotland during surveys conducted since 2003 and other recorded locations for *P. kernoviae* are New Zealand in 2006 and Ireland in 2008


United Kingdom Forestry Commission

Host Known to be Susceptible to *P. kernoviae*

Common Name	Scientific Name
Winters bark	*Drimys winterii*
European beech	*Fagus sylvatica*
Chilean hazelnut	*Gevina avellana*
Ivy	*Hedera helix*
Variegated holly	*Ilex aquifolium*
Tulip tree	*Liriodendron tulipifera*
Magnolia	*Magnolia* spp.
Holm oak	*Quercus ilex*
English oak	*Quercus robur*
Southern red oak	*Quercus falcata*
Northern red oak	*Quercus rubra*
Pieris	*Pieris formosa*
Cherry laurel	*Prunus laurocerasus*
Rhododenron	*Rhododendron ponticum* and others
Sweet Michelia	*Michelia doltsopa*
California Laurel	*Umbellularia californica*
Bilberry	*Vaccinium myrtillus*

Phytophthora kernoviae symptoms

Bleeding Bark Canker

Foliar Blight

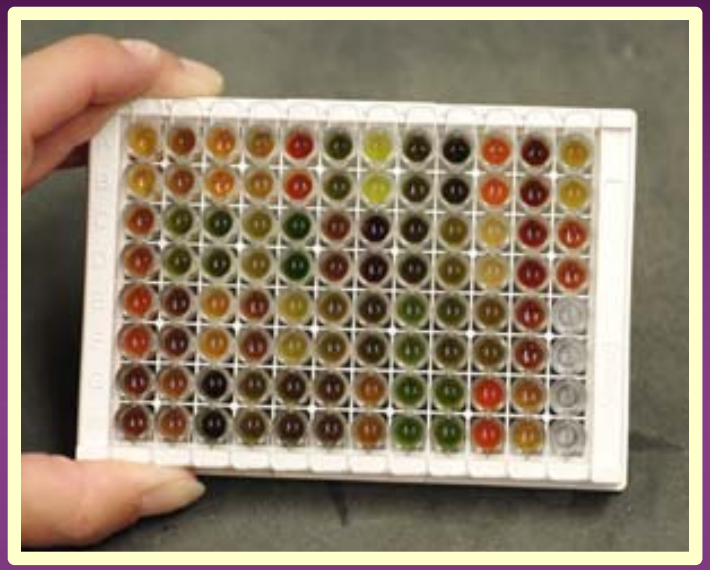
Twig Dieback

Objective #1-ELISA vs. Immunostrip

Tested NYS samples with both the ELISA and Immunostrip testing procedures.

•136 samples were tested both ways and 132 samples produced identical results.

•4 samples tested ELISA positive and Immunostrip negative, therefore, 3% produced different results.



Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

Objective #2-*P. kernoviae* PCR testing

Tested NYS samples using the *P. kernoviae* ITS1 and ITS2 real time PCR testing protocols.

71 samples were tested and all samples produced **negative** results, **no *P. kernoviae* found**.

All controls produced expected results…positive and negative test controls and internal controls testing for plant DNA presence and generic *Phytophthora* species presence.

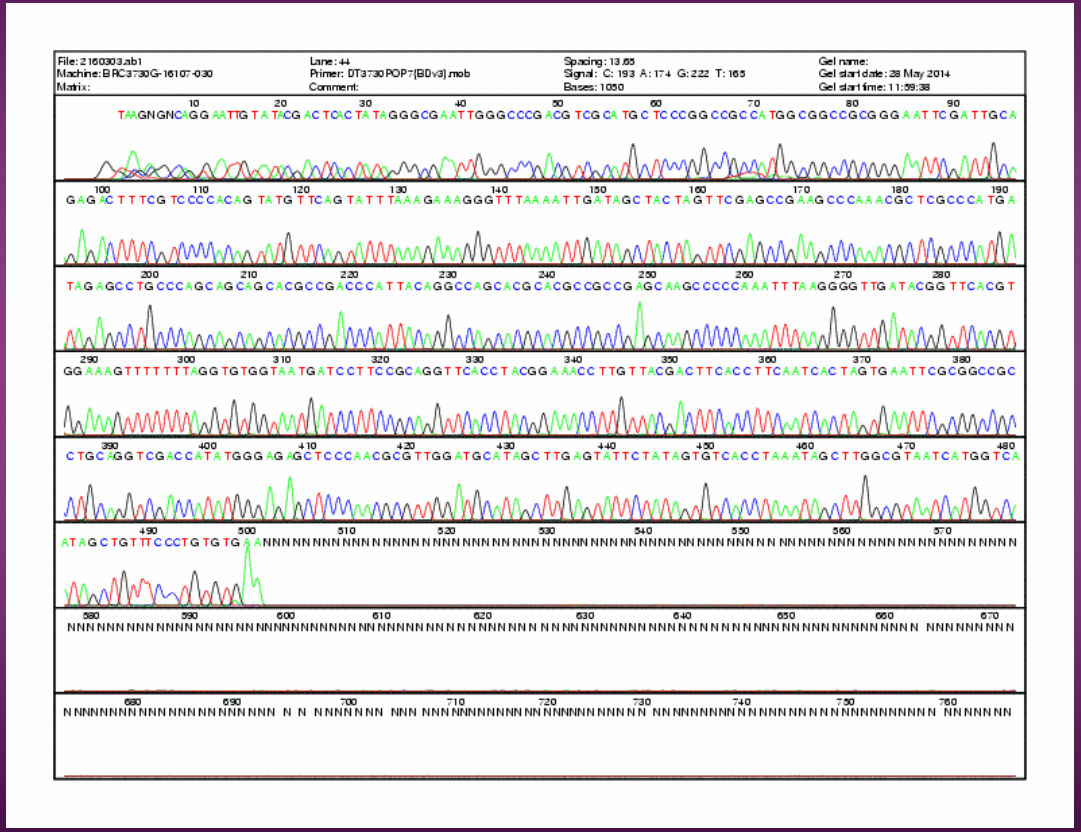
Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

Objective #3-Sequencing

In the process of sequencing all the NYS samples that were not positive for *P. ramorum*.

Preparing samples by cloning, performing PCR with generic Phytophthora species primers, and sequencing clean-up kits.

Should have results in the next few weeks.



Cornell University
Department of Plant Pathology
and Plant-Microbe Biology

Thank you!

Any Questions?

Cornell University
Department of Plant Pathology
and Plant-Microbe Biology