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Christmas Tree Survey for New Phytophthora Root Rot Diseases

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2014 State Capitol Christmas tree donated by Dennis & Kim Schoeneck, Pelican Lake, WI.

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DATCP Survey

- Response to growers reporting tree losses and 2010 Phytophthora sp. 'kelmania' find prompts survey.
- USDA Specialty Crop Block Grant and DATCP fund survey from 2011 to 2014.
- Christmas tree inspectors screen for problem fields during inspections.
- Collect trees and seedlings with symptoms.
- DATCP Plant Industry Bureau Laboratory diagnoses Phytophthora species.

Root collar rot on Fraser fir. Bark removed to expose discolored cambium.

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Christmas Tree Survey for New Phytophthora Root Rot Diseases

Survey Goals

- Identify new Phytophthora root rot species,
- fungus-like organisms that live in soil.
- How widespread are new species in WI?
- Which host trees are affected?
- Collaborate with research and extension.

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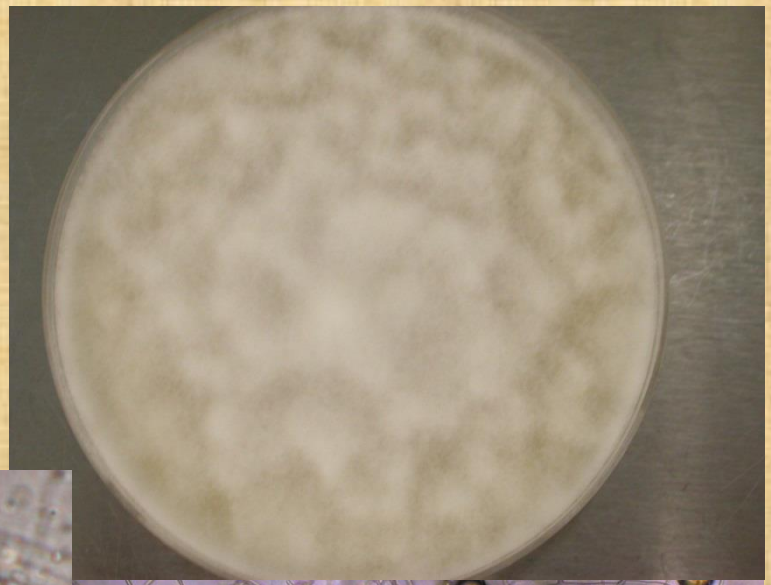
Phytophthora Root and Collar Rot

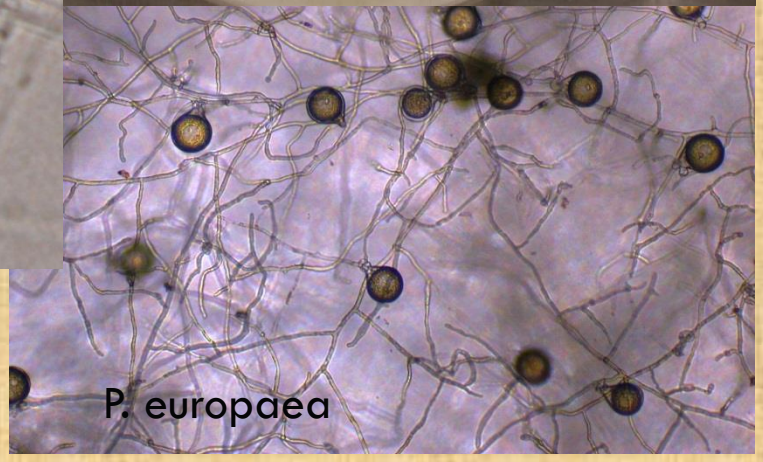
- Microscopically small fungi, Oomycetes
- Phytophthora causes root rots of tree seedlings and grown trees.
- Pythium causes root rot on seedlings only.
- Many host plants including flowers, shrubs, vegetables, soybeans and corn.

Fungal hyphae growing on a petri dish



P. sansomeana

Oogonium fruiting structure of *Phytophthora* under microscopy (400X magnification)



P. europaea

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Table 1. Christmas Tree Program Survey Statistics				
Year	**2011**	**2012**	**2013**	**2014**
Collection timeframe	9/26-10/14	8/16-10/29	9/15-10/31	9/8-10/17
Growers inspected	297	304	324	278
Growers sampled	**32**	**37 (26 new)**	**31 (23 new)**	**23 (10 new)**
Fields inspected	689	702	767	666
Fields sampled	**51 (7.4%)**	**58 (8.3%)**	**44 (5.7%)**	**31 (4.7%)**
Counties sampled	18	18 (8 new)	17 (4 new)	14 (3 new)

Total collected: 187 samples from 91 growers in 33 counties.

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Fraser fir infected with Phytophthora sansomeana /europaea

- Check root collar, trunk at soil line.
- Remove bark.
- Peel back layers of wood.
- Look for discoloration.
- Fine roots rooted off.

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Table 1. Tree host species, total number sampled	
	2010-2014
Balsam	**44**
Fraser	**115**
Canaan	4
Douglas	2
Korean	1
Pine	9
Spruce	12
Grand Total	**187**

Fraser fir was the most frequently sampled tree.

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Table 2. Number of Phytophthora infected samples for each tree host species/ total sample number of each tree host species

Tree host species	2010*	2011	2012	2013	2014	**2010-2014**
Balsam fir	0	**0/10**	**2/8**	**5/19**	**1/7**	**8/44 (18%)**
Fraser fir	1/1	**14/35**	**12/35**	**4/24**	**5/20**	**36/115 (31%)**
Canaan fir	1/1	0/1	1/2	0	0	2/4
Douglas fir	1/1	0	0	0	0/1	1/2
Korean fir	0	0/1	0	0	0	0/1
Pine	0	0/3	0/6	0	0	0/9
Spruce	0	0/1	0/7	0/1	0/3	0/12
Grand Total	3/3*	14/51 (27%)	15/58 (26%)	9/44 (20%)	6/31 (19%)	**47/187 (25%)**

* First detections of new Phytophthora species in 2010, prior to survey in 2011.

Fraser fir was the most root rot susceptible tree host.

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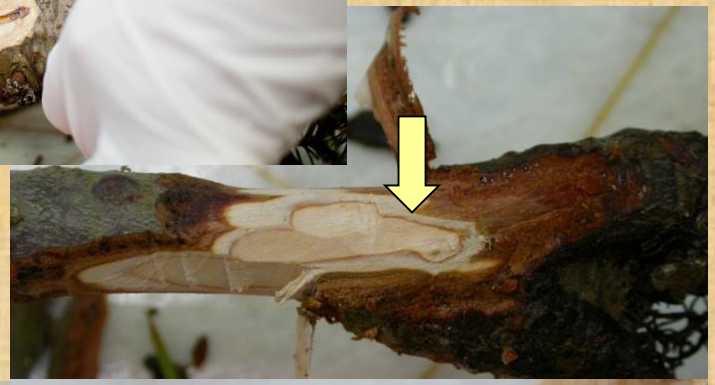
Diagnostic Methods

- Check for red-brown discolored cambium wood.
- Test fine roots of seedlings if no discolored wood is found.
- Extract genetic material from fungi in wood for PCR and DNA analysis.
- Culture fungi on growth media plates.

Checking for infected wood tissue



Isolating fungi out of wood chips in growth media.

Discolored wood tissue

Photos by David Brabender

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TTATCCACCATTATCTAGTGTACAAGCACACTCAGGACCT

How to DNA Barcode

Laboratory Methods

1. Extract Fungal DNA directly from wood.
2. Amplify DNA sequences of COI (mitochondrial Cytochrome oxidase I) regions by PCR.
3. Send amplification product to contract lab for sequencing.
4. Analyse sequence by comparing to known sequences on GenBank ®
   National Center of Biotechnology Information
5. Identify Phytophthora to species level.

Photos by David Brabender

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Table 3. Phytophthora species found on Wisconsin noble fir						
Phytophthora species	**2010***	**2011**	**2012**	**2013**	**2014**	**2010-2014**
P. europaea		**8**	**12**	**5**	**2**	**27**
P. sansomeana		**6**	**1**	**1**	**2**	**10**
P. sp. 'kelmania'	3	0	0	0	0	3
P. cactorum		0	1	2	1	4
P. megasperma		0	0	0	1	1
P. plurivora (syn. citricola)		0	1	1	0	2
Total number of samples with Phytophthora (%)	3	14 (27%)	15 (26%)	9 (20%)	6 (19%)	47 (25%)
Total number of samples tested.	3	51	58	44	31	187
Percent of **fields** infected with Phytophthora	NA	2.00%	2.10%	1.20%	0.90%	1.55%

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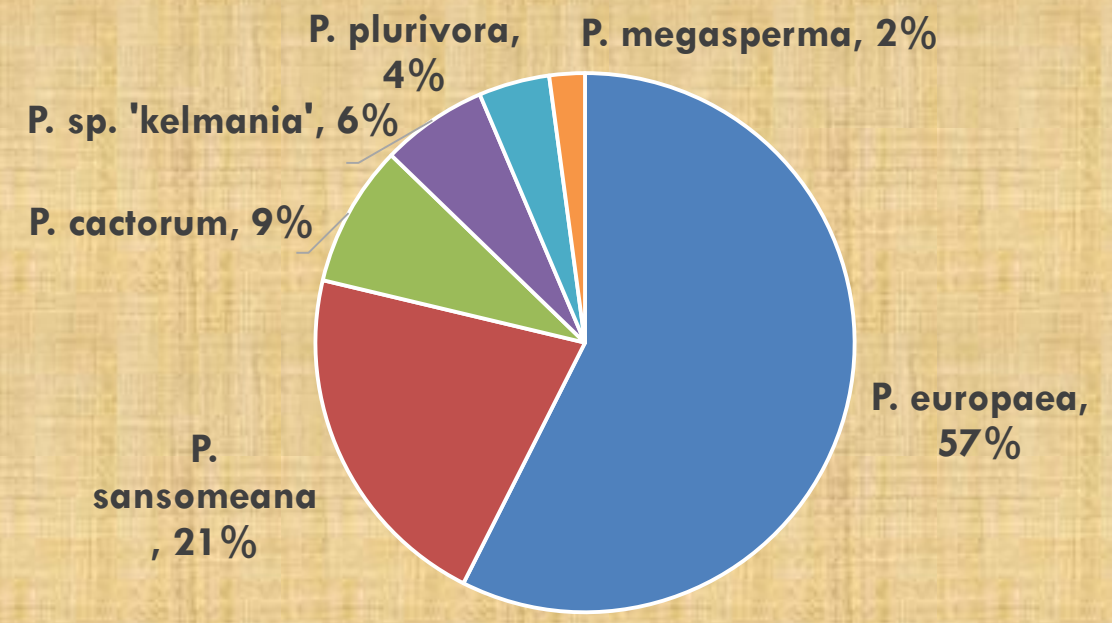
Phytophthora species on Christmas fir trees



Phytophthora europaea was the most common species.

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Phytophthora europaea

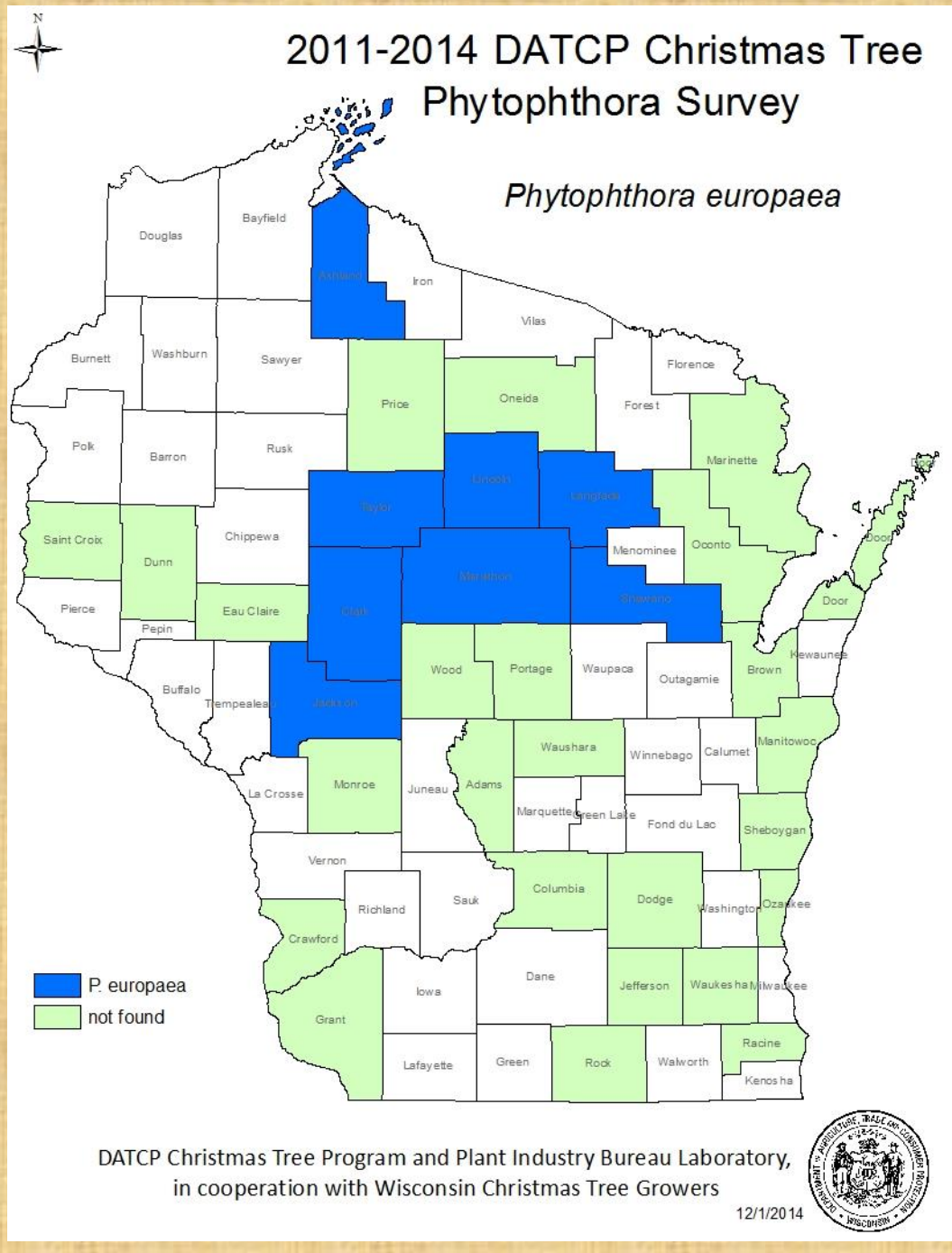
- First detection in Wisconsin in 2011.
- Wisconsin survey detected it on root collars of Balsam fir and Fraser firs.
- Literature reports it in forest soils around oak trees in Minnesota, West Virginia, Wisconsin and Pennsylvania (2006), Europe (2002).
- Weak pathogen of oak.

Oogonium of *Phytophthora europaea* under the microscope.

Phytophthora europaea

Detected in 8 Wisconsin counties:

- Ashland
- Clark
- Jackson
- Langlade
- Lincoln
- Marathon
- Shawano
- Taylor



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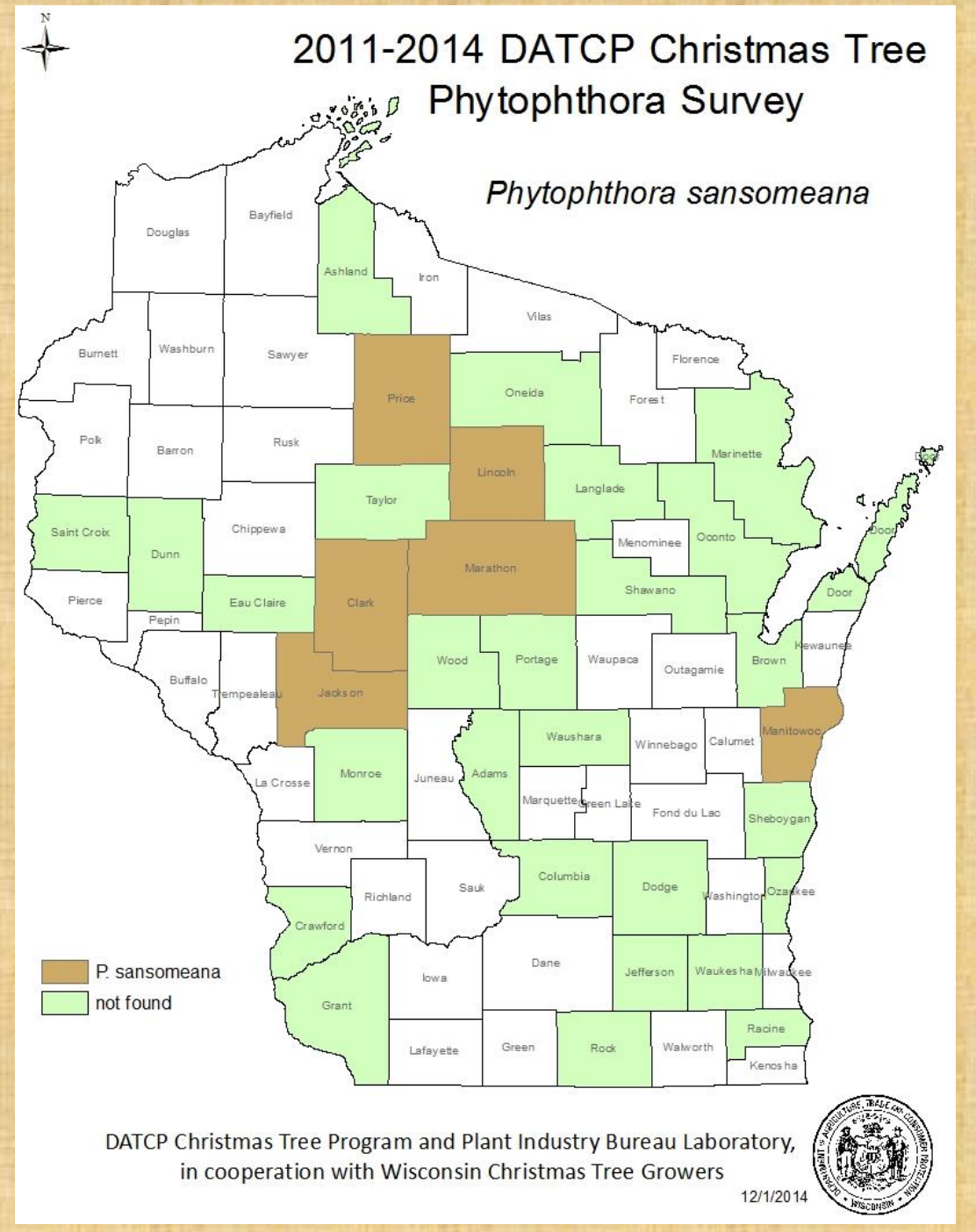
Phytophthora sansomeana

- Wisconsin survey detected it on root collar of Balsam fir and Fraser fir. First detection in 2011.
- Scientific Literature:

  Douglas fir (Oregon),
  corn (Ohio),
  soybean (Indiana),
  weeds in alfalfa fields (NY), (2009).



Phytophthora sansomeana

Detected in Christmas tree fields in 6 Wisconsin counties:

- Clark
- Jackson
- Lincoln
- Manitowoc
- Marathon
- Price

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Hosts of *Phytophthora sansomeana*

- Corn
- Soybean
- Weeds in alfalfa fields
- Christmas trees:
  on Balsam, Fraser fir,
  Douglas fir …..

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Phytophthora sansomeana

First detection on Wisconsin soybeans in 2012 and corn in 2013.

Phytophthora sansomeana and *Pythium* infecting fine and tap roots of soybean seedlings.

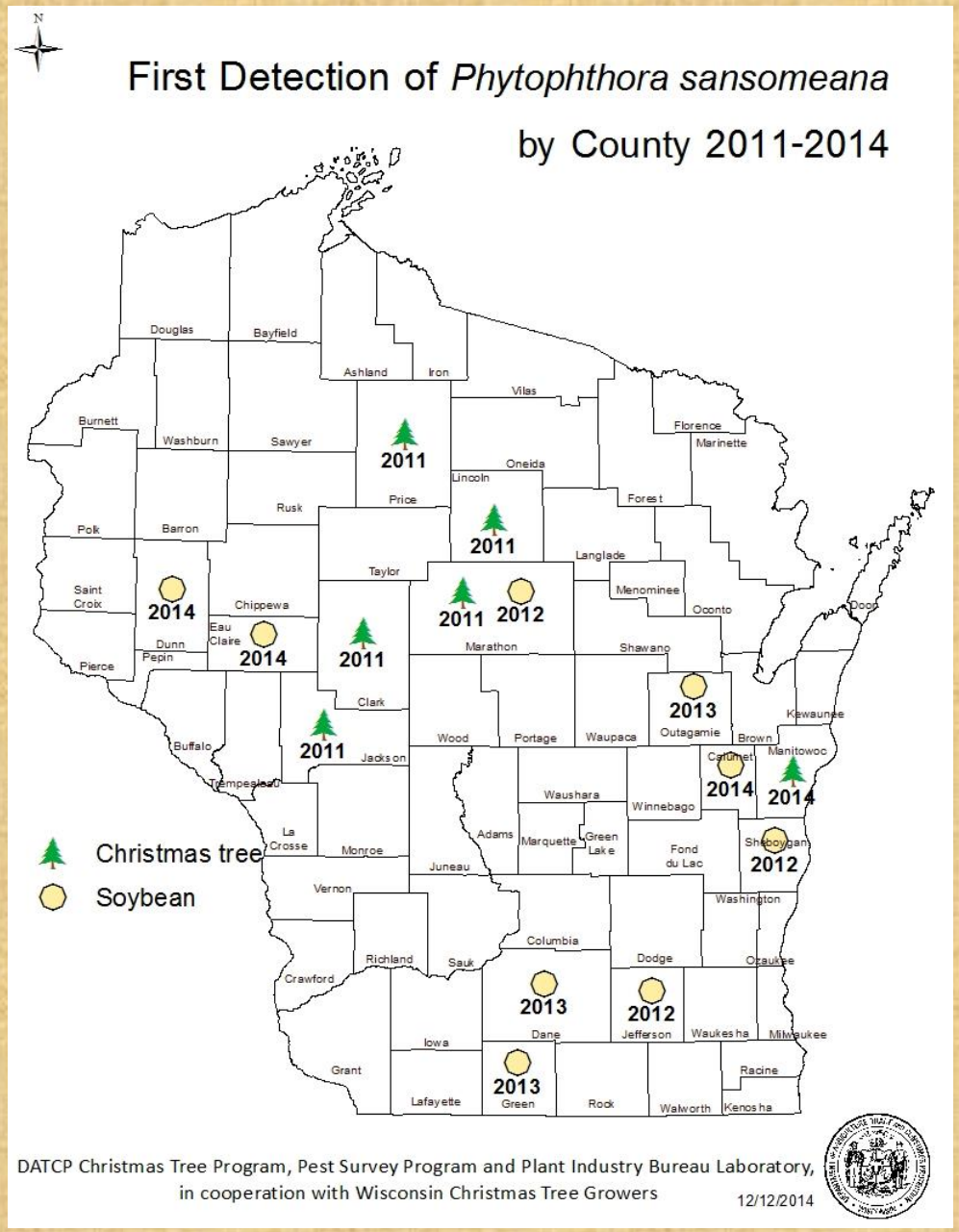
DATCP

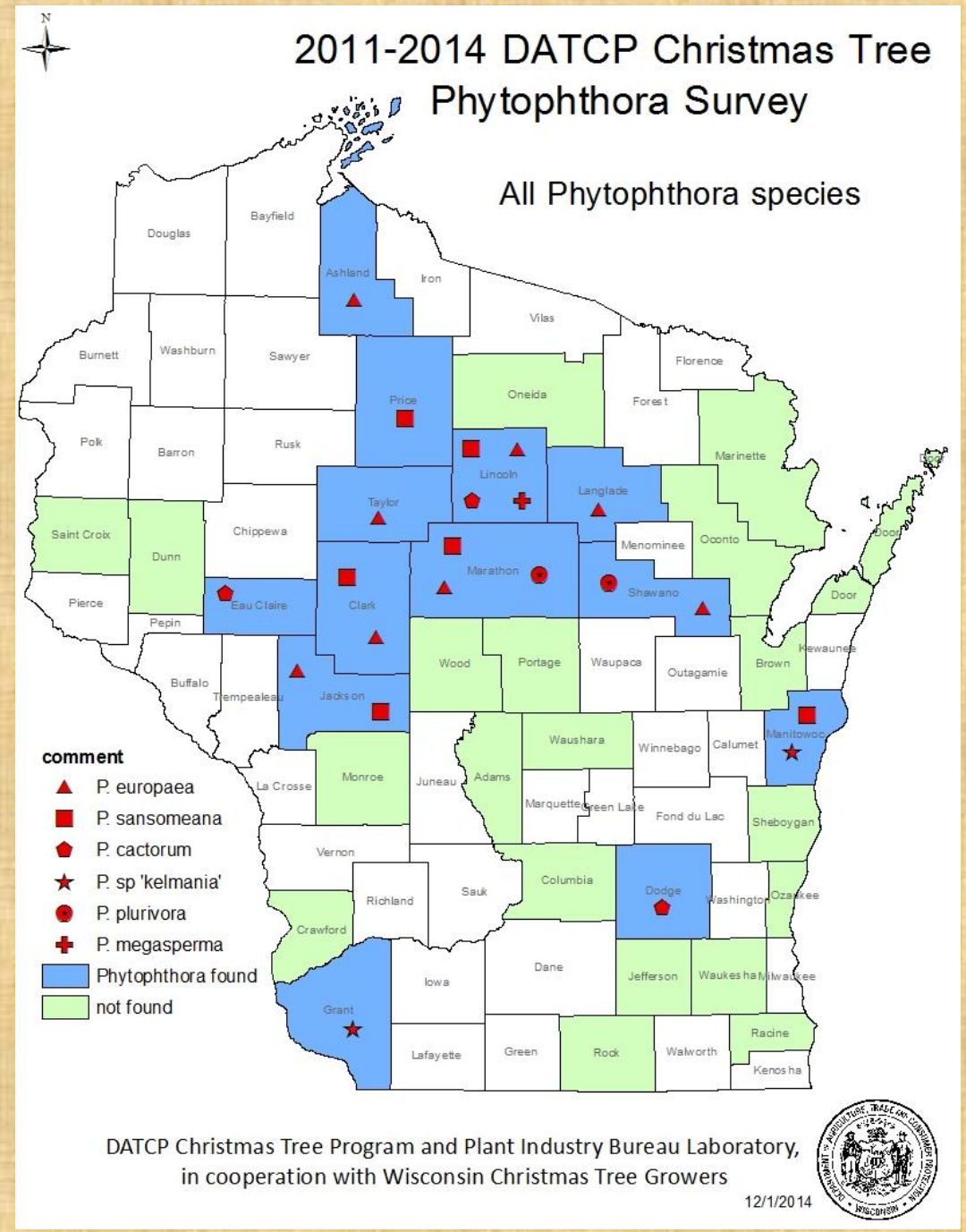
Prevalence of *Phytophthora sansomeana* in Wisconsin

On Soybeans / Christmas trees

- Calumet
- Clark
- Dane
- Dunn
- Eau Claire
- Green
- Jackson
- Jefferson
- Lincoln
- Manitowoc
- Marathon
- Outagamie
- Price
- Sheboygan

Present in 14 counties.





Phytophthora species

- 6 different Phytophthora species identified in
- 13 of 33 surveyed Wisconsin counties.
- 3 species are new to Wisconsin:
  - ***P. europaea***
  - ***P. sansomeana***
  - ***P. sp. 'kelmania'***
- 3 species known:
  - *P. cactorum*
  - *P. plurivora*
  - *P. megasperma*

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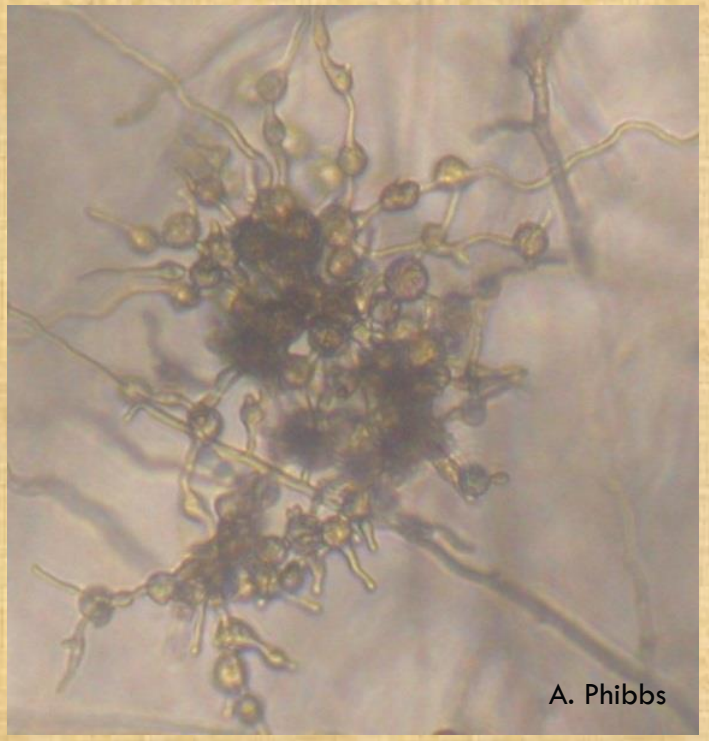
Phytophthora sp. 'kelmania'

- In Wisconsin found on root collar of Canaan fir, Fraser Fir, Douglas fir.
- First detection in 2010, Grant and Manitowoc Co.
- Scientific Literature: Fir, Spruce (North Carolina), Douglas fir, Gerbera and Coleus (Spain).



Characteristic hyphal swellings of *P. sp. 'kelmania'*

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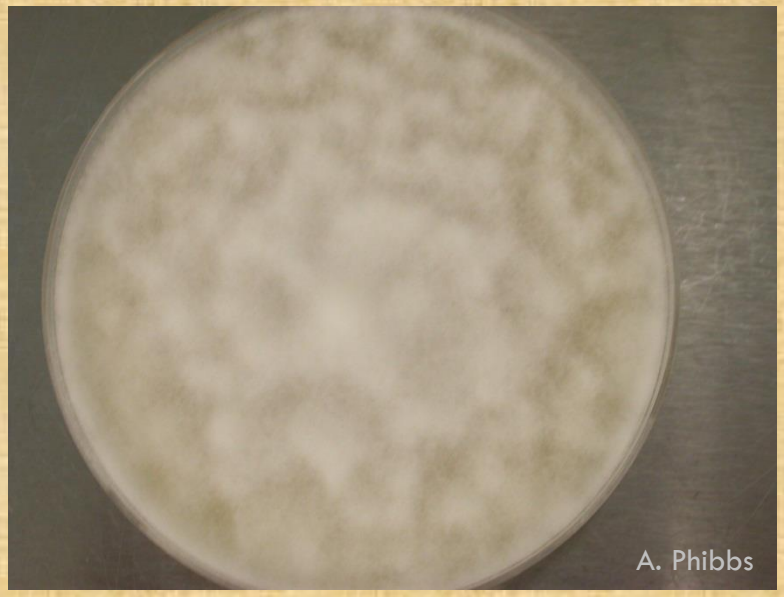
Research & Collaborations

- Dr. Gary Chastagner,
  Kathy McKeever
  Washington State University, WA.
- Dr. Gloria Abad, USDA APHIS
  Beltsville Laboratory, MD.
- Dr. Yilmac Balci, Univ. of Maryland
- Dr. Michael Coffey, Univ. of CA Riverside,
  World Phytophthora Collection
- Dr. Frank Martin, USDA ARS, Salinas CA
- Damon Smith,
  University of Wisconsin-Madison



Phytophthora sansomeana culture on rye agar.

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Preliminary loss estimates as of January 2015

- 51% average losses by *P. europaea* and *P. sansomeana*.

Host tree	Phytophthora species	Total Acres	% Loss
Balsam fir	*Phytophthora europaea*	40.0	25%
Balsam fir	*Phytophthora europaea*	15.0	13%
Balsam fir	*Phytophthora europaea*	1.0	100%
Balsam fir	*Phytophthora sansomeana*	25.0	90%
Fraser fir	*Phytophthora europaea*	65.0	18%
Fraser fir	*Phytophthora europaea*	40.0	80%
Fraser fir	*Phytophthora europaea*	7.0	29%
Fraser fir	*Phytophthora europaea*	7.0	35%
Fraser fir	*Phytophthora europaea*	1.0	33%
Fraser fir	*Phytophthora europaea*	1.0	100%
Fraser fir	*Phytophthora sansomeana*	70.0	43%
Fraser fir	*Phytophthora cactorum*	4.5	0.25%
Nova Scotia fir	*Phytophthora megasperma*	10.0	1%
Fraser fir	*Phytophthora plurivora*	20.0	15%

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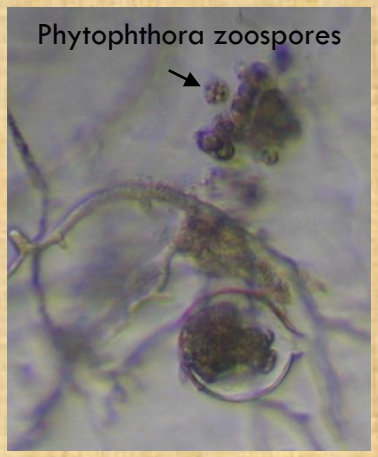
Disease management is root rot prevention

- Start with clean disease-free seedlings.
- Don't plant in sites with a history of Phytophthora root rot.

  Persistence of disease in soil is a long term disease management issue.
- Ensure that fields drain well, avoid heavy clay soils and areas where water pools. Improve drainage. *Phytophthora zoospores actively spread in water.*
- Don't irrigate with surface water, use well water.

  Some species of Phytophthora can survive in ponds and rivers.
- Avoid wounding roots, don't prune roots.

  Wounded roots attract Phytophthora zoospores.



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Root problems start at planting!

- Self-girdling roots,
- fine roots rotted off,
- root compaction,
- shallow lateral growth,
- J-roots.

Root girdling on Fraser fir.

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Root compaction

Year	Total tree samples	Number of tree samples with compacted roots	%
2011	51	21	41.2%
2012	58	31	53.4%
2013	44	22	50.0%
2014	31	19	61.3%
Total	**184**	**93**	**51.5%**

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J-roots on pine

Prevent root compaction

- Seedlings should be planted in a way that allows the roots to spread out and grow many fine roots.
- Fine roots are necessary to take up water and nutrients.
- Healthy root growth allows trees to better withstand drought conditions and root infections.

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Frost split on trunk of Arborvitae

- Frost splits likely due to early spring warm–up followed by a hard frost in April 2011.
- Killing branches or whole trees.

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Christmas Tree Survey

- Christmas trees in Central Sands counties suffered heavily from the drought in 2012.
- 25 Wisconsin counties were in the drought zone.
- Out of 446 fields inspected in the drought zone

  21.7% of fields were affected,

  10.3% had heavy losses. (DATCP Christmas Tree Program)
- These fields were not sampled for this survey because of the obvious impact of drought conditions.

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Armillaria rot root
white fungal fans
on root collar of Balsam fir,
bark removed.

Armillaria on Balsam fir.

Armillaria on Fraser fir

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Bluestain fungi

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Longhorn (Cerambycid) beetle gallery and larvae.

Root aphids

Prociphilus americanus

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Summary conclusions

- **91 growers in 33 counties surveyed.**
- **27 tree farms positive for Phytophthora.**
- **25% of tree samples were infected with Phytophthora**
- **Fraser fir are most susceptible but serious losses occur in Balsam fir, too.**
- **New species of Phytophthora are widely distributed in Christmas tree growing counties, except in central sands area.**
- ***P. sansomeana* causes root & collar rot.**
- ***P. europaea* possibly causing root & collar rot, more research needed.**

2014 State Capitol Christmas tree donated by Dennis & Kim Schoeneck, Pelican Lake, WI.

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http://pestsurvey.wi.gov/plantdisease/ornamentals.html

Thank you

Wisconsin Christmas Tree Growers
for participating!

Thank you

DATCP Inspectors

Brooke Sanneh, Sara Ott,
Konnie Jerabek, Christel Zillmer,
Lenny Weiss, Marcia Wensing,
Liz Meils, Greg Helmbrecht,
Ellen Hermanson, Adrian Barta,
Nick Clemens, John Domino.

DATCP and USDA Specialty Crop Block Grant Program

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TTATCCACCATTATCTAGTGTACAAGCACACTCAGGACCT

DNA Barcoding Rocks!

DNA Analysis for Identification of Phytophthora to species level.

LITERATURE

- "Phylogenetic relationships among Phytophthora species inferred from sequence analysis of mitochondrially encoded cytochrome oxidase I and II genes." F. N. Martin, P. W. Tooley. Mycologia, 95(2) 2003, pp. 269-284.

- "DNA Barcoding of Oomycetes with Cytochrome C Oxidase subunit 1 and internal transcribed spacer". Robideau et al, 19 authors. Mol. Ecol. Resource., 2011 Nov: 11(16) 1002-1011.
- "A Molecular Phylogeny of Phytophthora and Related Oomycetes"

  D. E. L. Cooke et al. ,*Fungal Genetics and Biology 30, 17–32 (2000)

  Based on Internal Transcribed Spacer (ITS) region of nuclear DNA.

- DNA sequencing by Functional Biosciences, Madison WI.