Wisconsin Department of Agriculture, Trade and Consumer Protection

Final Report 2015

Christmas Tree Survey for New Root Rot Diseases http://pestsurvey.wi.gov/

A survey of Wisconsin Christmas tree fields for new root rot diseases revealed six different species of Phytophthora on declining trees (*P. cactorum*, ***P. europaea***, *P. megasperma*, *P. plurivora*, ***P. sansomeana, P. sp. 'kelmania'***). Three of these species (in bold print) are new to Wisconsin. Phytophthora are soil-living fungus-like organisms that can cause root rot under wet conditions. The discovery of *Phytophthora sp. 'kelmania'*, in 2010, initiated a four year project with the goal to identify Phytophthora species affecting tree production in the state. The final tally shows Phytophthora was found in 13 of 33 surveyed Wisconsin counties, and 27 of 91 participating tree farms, causing total losses in some fields.

Methods

DATCP inspectors surveyed Christmas tree fields for Phytophthora root rot disease during annual inspections, carefully digging up affected trees, in fields where patches of dying or declining conifers were observed. Typical root rot symptoms are needles turning reddish-brown, starting with the bottom branches and progressing to the top. Often symptoms of Phytophthora root rot are not observed until the disease is quite advanced. Depending on tree size, two to six trees, were combined into one sample for each field including mildly symptomatic trees. The tree samples were transported to the DATCP's Plant Industry Laboratory for diagnosis. To check for root collar cankers, the bark was carefully peeled away from the lower trunk just above the soil line. The root collar and cambium wood was examined for caramel brown discoloration, which is typical for Phytophthora. Discolored wood pieces were cut out and prepped for DNA testing. If no root collar canker was observed, roots were examined and sampled if appropriate. This project utilized gene based laboratory methods such as PCR and sequence analysis which were essential in identifying Phytophthora to species level (5, 8, 10, 11, 13).

Results

From 2011 to 2014, 91 growers in 33 counties participated in this survey. We added more growers, fields and counties each year, to assess the prevalence of Phytophthora in Wisconsin Christmas tree growing areas. Table 1. shows the number of growers, fields and counties inspected and sampled each year.

Table 1. Christmas Tree Program Survey Statistics

Year	**2011**	**2012**	**2013**	**2014**	**Totals**
Collection timeframe	9/26-10/14	8/16-10/29	9/15-10/31	9/8-10/17	
Growers inspected	297	304	324	278	
Fields inspected	689	702	767	666	
Growers sampled	**32**	**37 (26 new)**	**31 (23 new)**	**23 (10 new)**	**91**
Fields sampled (% of total fields inspected)	**51 (7.4%)**	**58 (8.3%)**	**44 (5.7%)**	**31 (4.7%)**	**187***
Counties sampled	18	18 (8 new)	17 (4 new)	14 (3 new)	**33**

*Includes 3 fields sampled in 2010.

Over the 4 year survey, almost one third (29.7%) or 27 of all 91 participating tree farms had Phytophthora infected trees in at least one field. This is significant since the disease can easily spread within fields and to other sites by run-off water, and soil clinging to boots and equipment. On a sample basis 25% (47 of 187) of collected tree samples tested positive for Phytophthora. Other causes of tree decline were also diagnosed whenever possible. The state-wide average is 1.55% of the total fields inspected (tables 3). Although the percentage of Phytophthora infected fields visited during annual Christmas tree inspections appears to be small, this disease organism creates a long term problem in infected locations due to its ability to spread and remain viable in the soil for many decades.

Table 2. Number of Phytophthora (P. spp.) infected samples by tree host species. Positive testing samples / total sample number

Tree host species	2010*	2011	2012	2013	2014	**2010-2014**
Balsam fir	0	**0/10**	**2/8**	**5/19**	**1/7**	**8/44**
Fraser fir	1/1	**14/35**	**12/35**	**4/24**	**5/20**	**36/115**
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 project start in 2011.

Tree host species. Fraser fir (*Abies fraseri*) was the most root rot susceptible tree host of the seven different Christmas tree species inspectors collected. Fraser fir is also the most popular Christmas tree. Laboratory testing detected Phytophthora in 36 of 115 (31%) Fraser fir samples, followed by balsam fir (*Abies balsamea*) with 8 of 44 (18%) positive samples. A few other tree species also tested positive for Phytophthora such as Canaan fir (*Abies balsamea var. phanerolepis*), Nova Scotia balsam fir (*Abies balsamea x fraseri*) and Douglas fir (*Pseudotsuga menziesii*). Spruce (*Picea spp.*) and pine (*Pinus spp.*) were checked as well but tested negative for Phytophthora.

Phytophthora species. Table 3 lists the six distinct species of Phytophthora identified during this survey. Three species were new to tree production in Wisconsin: *P. europaea* and *P. sansomeana*, which were both detected on Fraser and balsam fir, and *P. sp. 'kelmania'* on Canaan, Fraser and Douglas fir. The next three species are known to be present in this state. *P. cactorum* was found on Canaan, Fraser and balsam fir, *P. plurivora* syn. *P. citricola* on Fraser fir and *P. megasperma* on Nova Scotia balsam fir. All three are capable of infecting a variety of plant hosts, including decidous shrubs, trees, and vegetables crops.

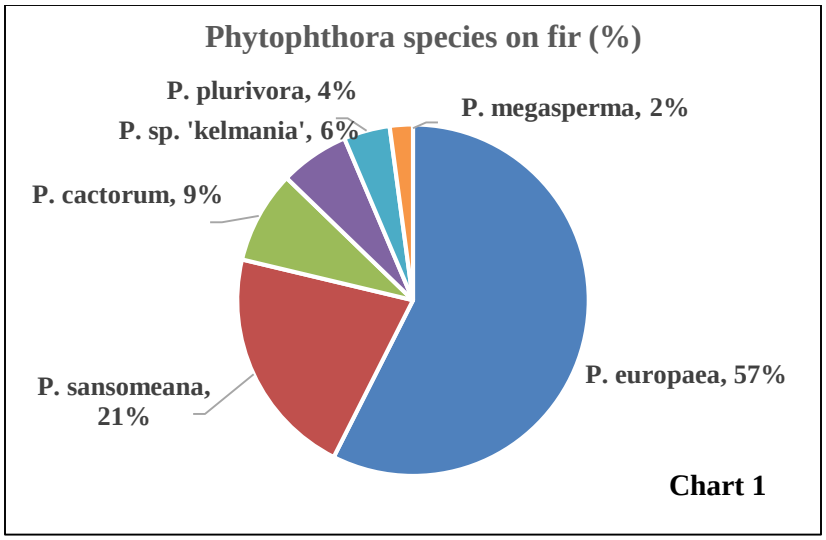

Chart 1 shows the most frequently found Phytophthora species was *P. europaea* with 27 of 47 (57%) total detections followed by *P. sansomeana* with 10 of 47 (21%). All other species were found only occasionally between 1 to 4 times of 47 (2-9%) total samples tested.

Table 3. Phytophthora species found in 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
Number of samples testing positive for Phytophthora	3	14/51 (27%)	15/58 (26%)	9/44 (20%)	6/31 (19%)	47/187 (25%)
Phytophthora positive testing field / total fields inspected	3/3	14/689 (2.0%)	15/702 (2.1%)	9/767 (1.2%)	6/666 (0.9%)	47/2824 1.55%

Information about new Phytophthora species

P. europaea was first reported in European forest soils around oak trees, and has since been collected from soil samples in oak forests in Minnesota, Ohio, Pennsylvania, West Virginia, and Wisconsin (3, 4, 7). It is considered a weak pathogen of oak. This survey found 22 Fraser and 5 balsam fir samples infected with *P. europaea*. It was first isolated in Wisconsin in 2011. It was found on the root collar and also on roots. Our observations indicate that it may be the primary cause of tree death, although more research is needed to prove pathogenicity on fir.

P. sansomeana is known to cause root rot on fir trees, especially Fraser fir. It was detected on 9 Fraser and 1 balsam fir sample during our survey. *P. sansomeana* has been reported on Douglas fir in Oregon and on several weed species in New York (6). It is a new pathogen of corn (Ohio)

and soybean (Indiana) in the Midwest. In Wisconsin we found *P. sansomeana* for the first time on fir in 2011, soybean in 2012 and on corn in 2013.

The discovery of *P. sp.'kelmania'* on Canaan, Fraser and Douglas fir in Grant and Manitowoc counties in 2010 lead to this survey project. This species has previously been reported on fir and spruce in North Carolina and on Gerbera and Coleus in Spain (1, 2, 9).

It is noteworthy that *P. cinnamomi*, the species most destructive on Fraser fir in its native Appalachian Mountain range and a very aggressive pathogen on many hosts, was not detected in Wisconsin and as far as we know does not occur in this state.

Prevalence in Wisconsin

The following maps show the counties where each Phytopthora species was detected. Phytophthora root and collar rot was present in most Christmas tree producing counties, except for the central sands where soils drain better, avoiding the water logged conditions that are favorable to these water molds.

During four years of survey *P. europaea* was found on Christmas tree farms in 8 counties (Ashland, Clark, Jackson, Langlade, Lincoln, Marathon, Shawano and Taylor), (figure 1). *P. sansomeana* was detected on fir trees in 6 counties (Clark, Jackson, Lincoln, Manitowoc, Marathon and Price), (figure 2). Figure 3 highlights all counties where Phytophthora species were found during this survey, broken down by species. During this survey *P. sp. "kelmania"* was found in Grant and Manitowoc counties. *P. cactorum* was documented in Dodge, Eau Claire and Lincoln counties; *P. plurivora* in Marathon and Shawano Cos, *P. megasperma* in Lincoln Co. The last three species are expected to be present in all counties and on a variety of crops and ornamentals.

Figure 4 combines first county detections of *P. sansomeana* from 2011 to 2014. It was found on fir trees in six and on soybean in nine Wisconsin counties, bringing the total number of Wisconsin counties with detections to 14. The soybean survey was part of the Cooperative Agricultural Pest Survey Program conducted by the authors.

Other biotic and abiotic problems affecting Christmas trees

Several other diseases, pests and abiotic problems were diagnosed on Christmas trees submitted for this project. Armillaria, cytospora, diaporthe/phomopsis, cerambycid beetle, white pine weevil and root aphid caused field symptoms similar to Phytophthora root rot. Blue stain fungi and bark beetle, frost injury to tips and winter kill were observed as well. Rhizosphaera needle cast, sclerophoma shoot blight, and spidermite injury were regularly diagnosed on fir and spruce.

The ability of conifers to maintain a green habit for months after death, which is a desirable characteristic for its purpose as a Christmas trees, can mask a problem for a long time. One problem that often goes undetected is root compaction. Poor root development is often the result of inadequate planting methods. Shallow lateral, j-shaped, fan-shaped root development and self-girdling roots were observed in 51.5% of collected samples. Root compaction adds to tree stress by limiting water and nutrient uptake. It leaves trees more susceptible to diseases, pests and less able to survive during drought conditions.

Over the course of this survey Wisconsin experienced unusually extreme weather patterns. Wisconsin Crop Progress reports documented growing conditions as varied as flooding, drought, heat and record cold (12). An early spring in 2011 with a sudden hard frost in April caused frost splits, killing branches and whole trees. Spring flooding occurred in 2010, 2013 and 2014 causing water logged soils and prime conditions for water molds. Christmas trees in the Central Sands counties were spared from flooding but suffered severely under the 2012 drought. 21.7% of 446 fields inspected in the drought zone were affected. 10.3% suffered heavy losses. These fields were not sampled for the survey because of the obvious impact of the drought. Record cold during the winter of 2013/2014 killed less cold hardy tree species like Fraser fir.

Loss estimates

Growers were asked to estimate losses for their fields that tested positive for *Phytophthora.* Table 4 reports percentage loss of acres sorted by host tree and Phytophthora species for each reported field. 14 of 27 growers responded to this question. The average loss estimate from *P. europaea* and *P. sansomeana* infected fields was 51%.

Table 4. Loss Estimates

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

Collaborations

Culture isolates of the new Phytophthora species were placed in national collections, such as World Phytophthora Collection (Dr. Michael Coffey, University of California Riverside), the USDA APHIS Beltsville Laboratory, (Dr. Gloria Abad, Maryland) and shared with researchers: Dr. Frank Martin, USDA ARS Salinas CA, Dr. Yilmac Balci at the University of Maryland, MD,

Dr. Gary Chastagner and Kathy McKeever, at Washington State University. Dr. Chastagner's research focusses on finding root rot resistant fir species for Christmas tree production.

University of Wisconsin-Madison Plant Diagnostic Clinic's Dr. Brian Hudelson and Ann Joy developed a factsheet on Phytophthora root rot diseases of Christmas trees for growers that includes disease management information. The factsheet is be posted on University of Wisconsin-Extension and DATCP websites and be made available in hard copy at Christmas tree producer's conventions.

References

1. Abad, Z. G., Abad, J. A., Creswell, T. (2002). Phytopathology 92 (6 SUPPL.): S1. "Advances in the integration of morphological and molecular characterization in *Phytophthora* genus: The case of *P. kelmania* and other putative new species."
2. Abad, Z.G., Abad, J.A., Creswell, T., (2006) Phytopathology 96(suppl.):S1."Species of *Phytophthora* and *Pythium* identified in a long term collection from North Carolina."
3. Balci Y., et.al. (2006) Plant Dis. 90:827."First Report of *Phytophthora europaea* in Oak Forests in the Eastern and North-Central United States".
4. Balci Y., Balci S., MacDonald W. L., Gottschalk K. W., (2008) For. Path. 38, pp. 320–331. "Foliar susceptibility of eastern oak species to *Phytophthora* infection".
5. Cooke D. E. L. et al. (2000) Fungal Genetics and Biology 30, 17–32. "A Molecular Phylogeny of *Phytophthora* and Related Oomycetes".
6. Hansen E. M., Wilcox W. F., Reeser P. W., Sutton W., (2009) Mycologia, 101(1), pp.129-135). "*Phytophthora rosacearum* and *P. sansomeana*, new species segregated from the *Phytophthora megasperma* ''complex''.
7. Jung, T., Hansen, E.M., Winton, L., Osswald, W., and Delatour, C. (2002) Mycol. Res. 106 pp. 397-411. "Three new species of *Phytophthora* from European oak forests."
8. Martin F. N., Tooley P. W., (2003). Mycologia, 95(2), pp. 269–284. "Phylogenetic relationships among *Phytophthora* species inferred from sequence analysis of mitochondrially encoded cytochrome oxidase I and II genes."
9. Moralejo, E., Pérez-Sierra, A.M., Álvarez, L. A., Belbahri, L., Lefort, F., Descals, E. (2009). Plant Pathology 58:100-110. "Multiple alien *Phytophthora* taxa discovered on diseased ornamental plants in Spain."
10. Park J., et.al. (2008) Plant Disease Vol. 92, Number 6, pp.966-972. "*Phytophthora* Database: A Forensic Database Supporting the Identification and Monitoring of *Phytophthora*."
11. Robideau et al. (2011) Mol. Ecol. Resource., Nov: 11(16) 1002-1011. "DNA Barcoding of Oomycetes with Cytochrome C Oxidase subunit 1 and internal transcribed spacer".
12. Wisconsin Crop Progress & Conditions. USDA-NASS Reports (2010-2014).
13. Sequencing Services by Functional Biosciences, Inc., Madison WI.

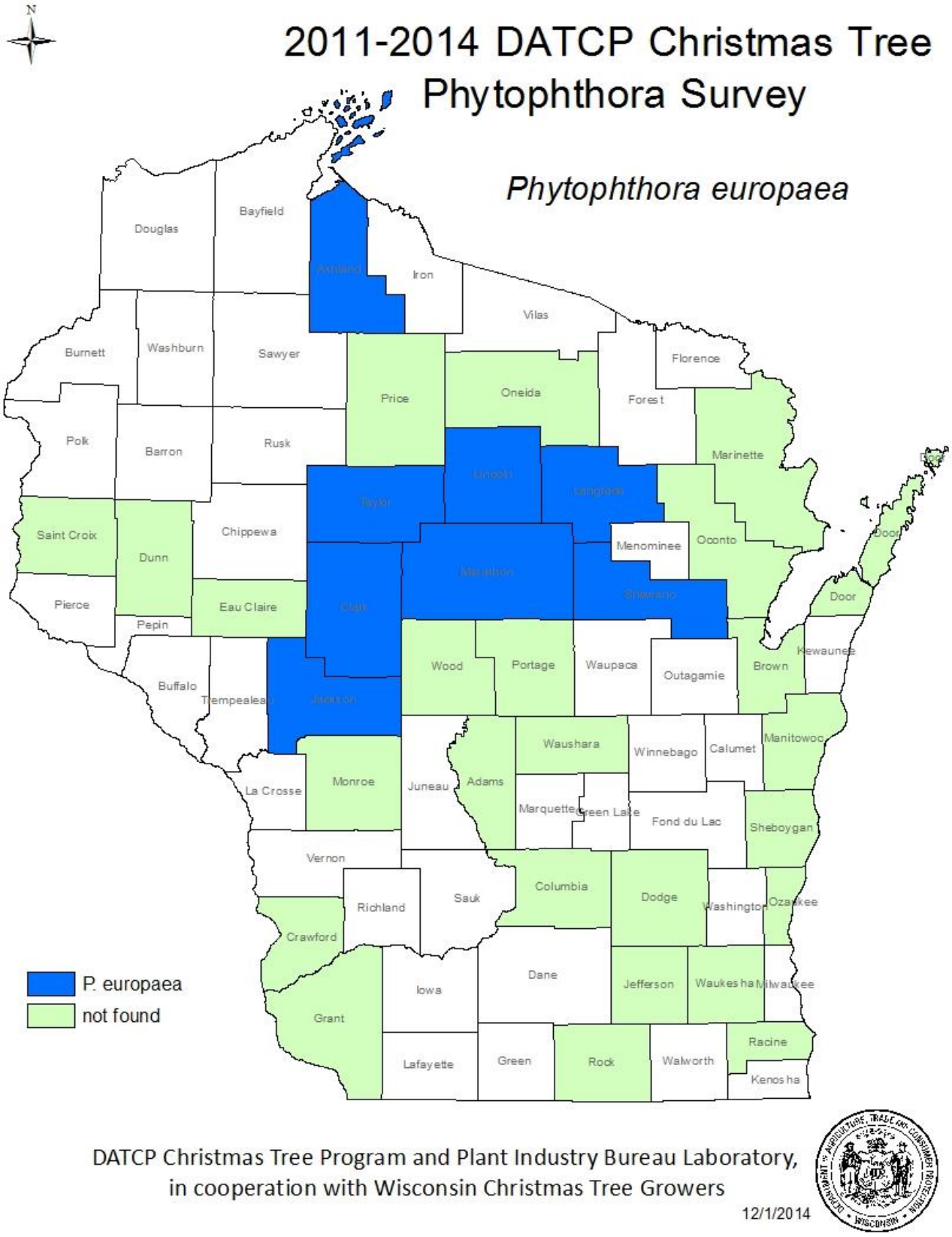
2011-2014 DATCP Christmas Tree
Phytophthora Survey
Phytophthora europaea
N
Douglas
Bayfield
Ashland
Iron
Vilas
Florence
Burnett
Washburn
Sawyer
Price
Oneida
Forest
Marinette
Polk
Barron
Rusk
Taylor
Lincoln
Langlade
Oconto
Saint Croix
Dunn
Chippewa
Menominee
Door
Pierce
Pepin
Eau Claire
Clark
Marathon
Shawano
Door
Buffalo
Trempealeau
Jackson
Wood
Portage
Waupaca
Outagamie
Brown
Kewaunee
La Crosse
Monroe
Juneau
Adams
Waushara
Winnebago
Calumet
Manitowoc
Marquette
Green Lake
Fond du Lac
Sheboygan
Vernon
Richland
Sauk
Columbia
Dodge
Washington
Ozaukee
Crawford
Dane
Jefferson
Waukesha
Milwaukee
Grant
Iowa
Lafayette
Green
Rock
Walworth
Racine
Kenosha
P. europaea
not found
DATCP Christmas Tree Program and Plant Industry Bureau Laboratory,
in cooperation with Wisconsin Christmas Tree Growers
12/1/2014

Figure 1

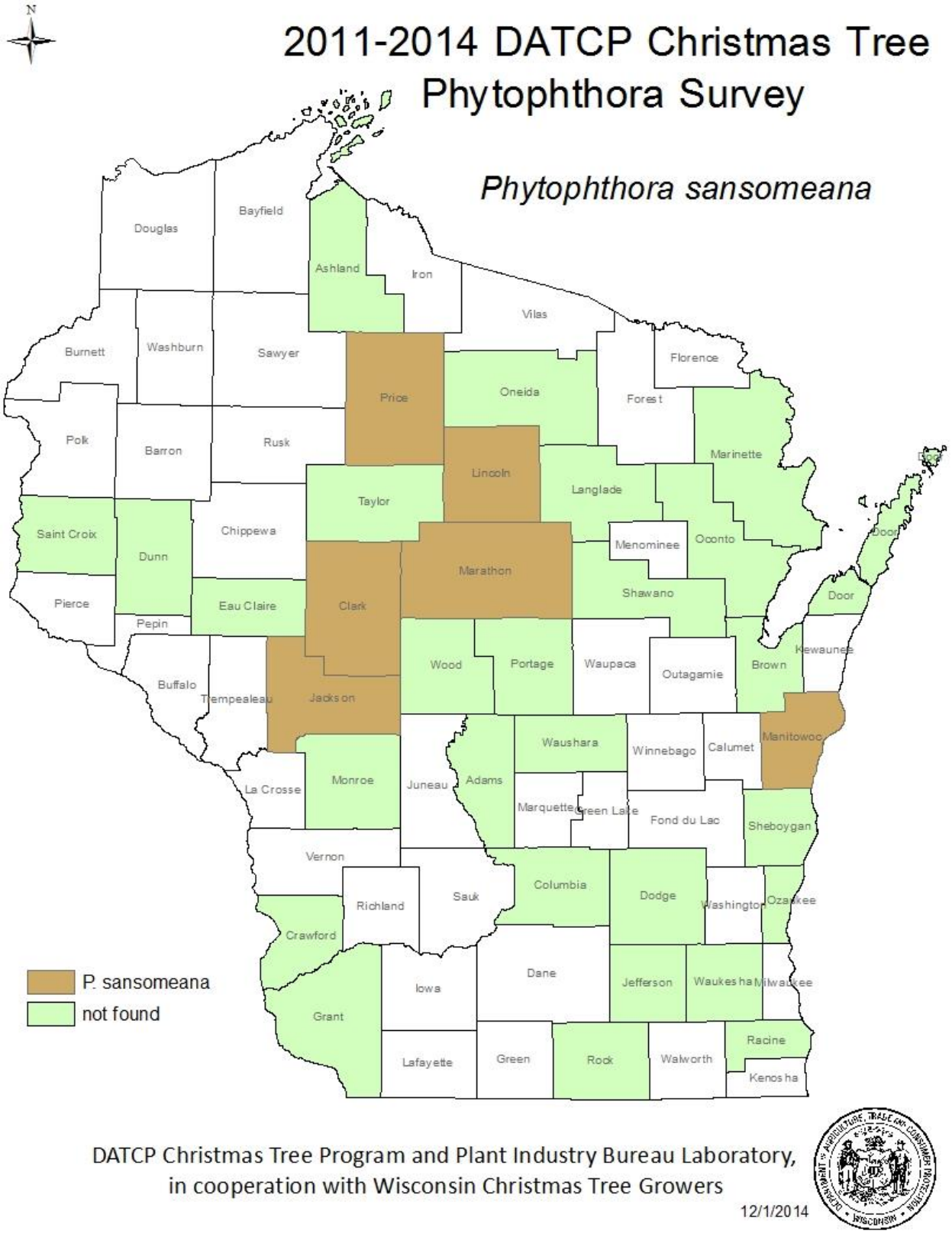
2011-2014 DATCP Christmas Tree Phytophthora Survey
Phytophthora sansomeana
N
Douglas
Bayfield
Ashland
Iron
Vilas
Florence
Burnett
Washburn
Sawyer
Price
Oneida
Forest
Marinette
Polk
Barron
Rusk
Lincoln
Langlade
Taylor
Saint Croix
Dunn
Chippewa
Menominee
Oconto
Door
Marathon
Shawano
Pierce
Eau Claire
Clark
Pepin
Wood
Portage
Waupaca
Outagamie
Brown
Kewaunee
Door
Buffalo
Trempealeau
Jackson
Waushara
Winnebago
Calumet
Manitowoc
La Crosse
Monroe
Juneau
Adams
Marquette
Green Lake
Fond du Lac
Sheboygan
Vernon
Columbia
Dodge
Washington
Ozaukee
Richland
Sauk
Crawford
Dane
Jefferson
Waukesha
Milwaukee
Iowa
Grant
Racine
Lafayette
Green
Rock
Walworth
Kenosha
P. sansomeana
not found
DATCP Christmas Tree Program and Plant Industry Bureau Laboratory,
in cooperation with Wisconsin Christmas Tree Growers
12/1/2014

Figure 2

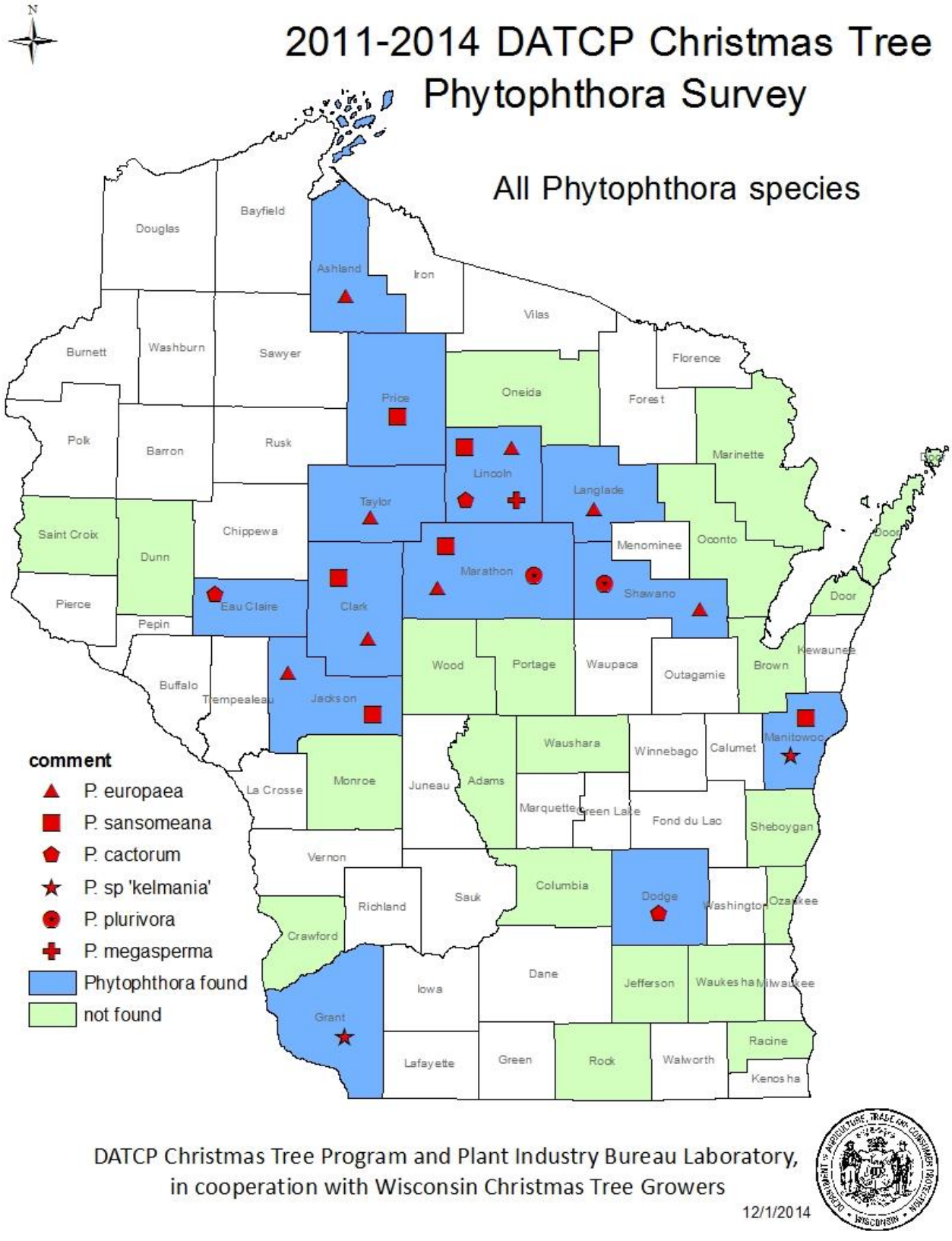
2011-2014 DATCP Christmas Tree Phytophthora Survey
All Phytophthora species
N
Douglas
Bayfield
Ashland
Iron
Vilas
Florence
Burnett
Washburn
Sawyer
Price
Oneida
Forest
Marinette
Polk
Barron
Rusk
Lincoln
Langlade
Taylor
Saint Croix
Dunn
Chippewa
Menominee
Oconto
Door
Pierce
Pepin
Eau Claire
Clark
Marathon
Shawano
Door
Kewaunee
Buffalo
Trempealeau
Jackson
Wood
Portage
Waupaca
Outagamie
Brown
Manitowoc
Waushara
Winnebago
Calumet
La Crosse
Monroe
Juneau
Adams
Marquette
Green Lake
Fond du Lac
Sheboygan
Vernon
Columbia
Dodge
Washington
Ozaukee
Richland
Sauk
Crawford
Dane
Jefferson
Waukesha
Milwaukee
Grant
Iowa
Lafayette
Green
Rock
Walworth
Racine
Kenosha
comment
P. europaea
P. sansomeana
P. cactorum
P. sp 'kelmania'
P. plurivora
P. megasperma
Phytophthora found
not found
DATCP Christmas Tree Program and Plant Industry Bureau Laboratory,
in cooperation with Wisconsin Christmas Tree Growers
12/1/2014

Figure 3

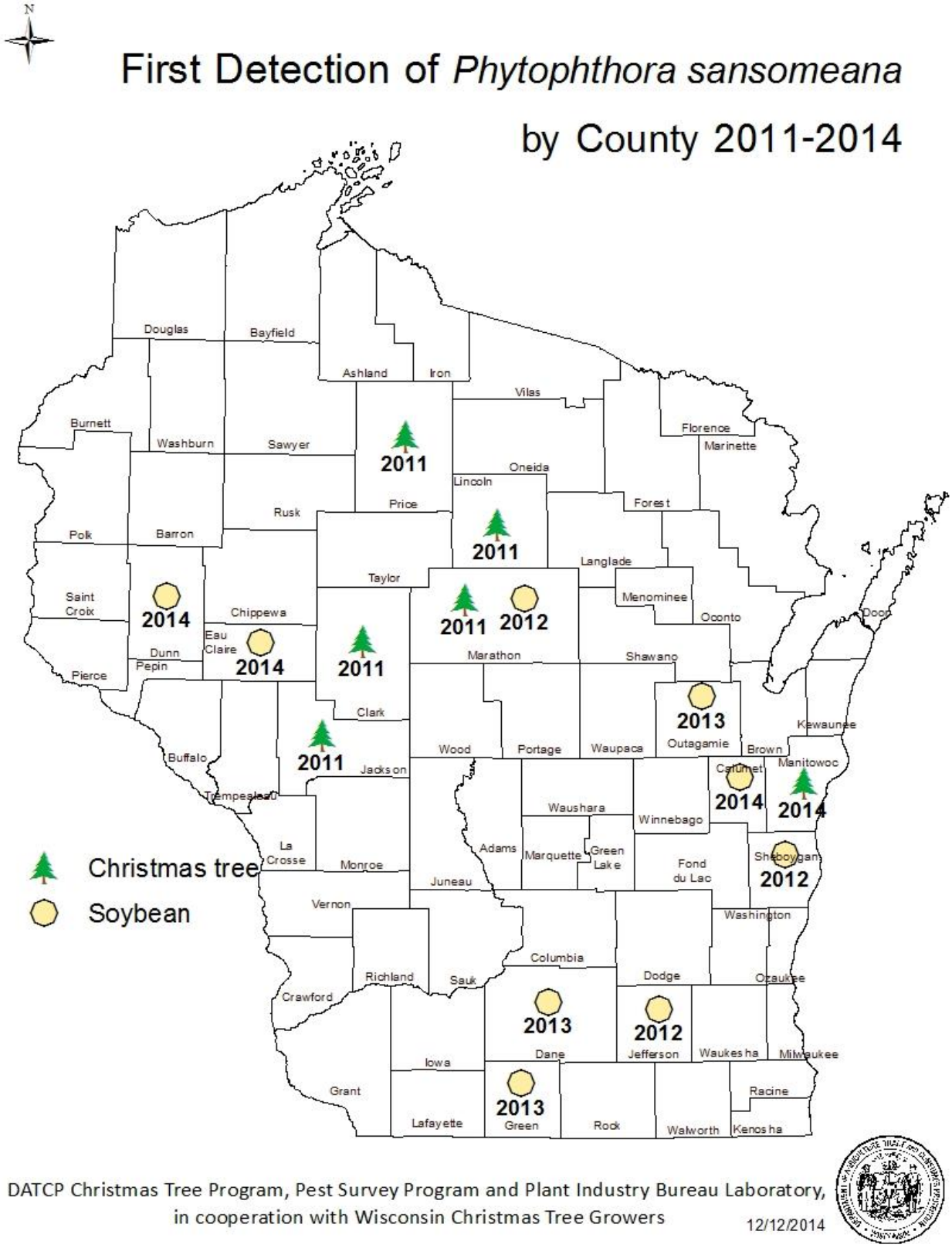
First Detection of *Phytophthora sansomeana*
by County 2011-2014
N
Douglas
Bayfield
Ashland
Iron
Vilas
Florence
Marinette
Burnett
Washburn
Sawyer
2011
Oneida
Lincoln
Price
Forest
Rusk
Polk
Barron
2011
Langlade
Taylor
Saint Croix
2014
Chippewa
2011
2012
Menominee
Oconto
Door
Eau Claire
2014
Dunn
Pepin
2011
Marathon
Shawano
Pierce
2013
Outagamie
Kewaunee
Clark
Buffalo
2011
Wood
Portage
Waupaca
Brown
Manitowoc
Jackson
Calumet
2014
2014
Trempealeau
Waushara
Winnebago
La Crosse
Adams
Marquette
Green Lake
Fond du Lac
Sheboygan
2012
Monroe
Juneau
Vernon
Washington
Columbia
Richland
Sauk
Dodge
Ozaukee
Crawford
2013
2012
Dane
Jefferson
Waukesha
Milwaukee
Iowa
Grant
2013
Racine
Lafayette
Green
Rock
Walworth
Kenosha
Christmas tree
Soybean
DATCP Christmas Tree Program, Pest Survey Program and Plant Industry Bureau Laboratory,
in cooperation with Wisconsin Christmas Tree Growers
12/12/2014

Figure 4