Wisconsin Department of Agriculture, Trade and Consumer Protection

Wisconsin Pest Survey Report

PYTHIUM SPECIES ASSOCIATED WITH SOYBEAN SEEDLINGS

The Wisconsin Department of Agriculture, Trade and Consumer Protection Pest Survey and Plant Industry Bureau Laboratory conducted disease surveys on soybeans from 2011 until 2020. This report documents *Pythium* species detected in soybean seedling roots. Soybeans in the early vegetative stages are often affected by this fungus-like organism, in a group called water molds or oomycetes. Pythium is most aggressive on early vegetative stages of soybeans. It can cause seed rot, damping-off and non-lethal root infections which can reduce plant growth and yield. Soybean seedlings were also infected with several other closely related oomycetes.

The survey shows that *Pythium* was present in almost all soybean fields (Table 1) and that a great variety of species were found in root samples (Table 2). As of 2020, 23 different Pythium species have been identified. A summary of Phytophthora species identified during our survey can be found on this website under "Early Season Soybean Root Rot Survey".

Table 1 Year	Survey Dates	Total Fields Surveyed	Fields with Pythium
2011	6-14 to 7-14	15	13 (87%)
2012	5-29 to 7-2	49	49 (100%)
2013	6-17 to 7-18	52	49 (94%)
2014	6-6 to 7-16	57	57 (100%)
2015	6-2 to 6-30	50	50 (100%)
2016	6-7 to 7-13	53	NA
2017	6-9 to 6-30	55	53 (96%)
2018	6-11 to 7-6	54	52 (96%)
2019	6-21 to 7-10	52	52 (100%)
2020	6-8 to 7-3	50	43 (86%)

Includes two corn fields in 2013 and four corn fields in 2014.

Methods - DATCP plant pathologists collected soybean seedlings from up to 57 fields, when soybeans were in the vegetative to early reproductive stages. From each randomly chosen field, 20 seedlings were carefully dug up, selecting symptomatic plants or plants from areas prone to flooding or soil compaction. The combined sample from each field was diagnosed by the Plant

Table 2. Pythium species present in soybean roots	Percent infested / total surveyed fields		
P. acanthicum	1.0%		
P. afertile	0.2%		
P. aphanidermatum	1.2%		
P. aristosporum	0.5%		
P. arrhenomanes	9.5%		
P. attrantheridium*	6.5%		
P. conidiophorum	4.4%		
P. heterothallicum	4.8%		
P. hordeum	0.2%		
P. inflatum	3.7%		
P. insidiosum	0.2%		
P. intermedium	0.2%		
P. irregulare	5.1%		
P. kunmingense	0.5%		
P. minus	0.2%		
P. paroecandrum	0.2%		
P. recalcitrans*	9.2%		
P. salinum	1.6%		
P. sulcatum	1.6%		
P. sylvaticum	21.4%		
P. torulosum	3.5%		
P. ultimum	2.5%		
P. violae	0.2%		
P. spp.	16.4%		
Cumulative count 2011-2020.			

^{*:} Recently described species, bold italic. pathogenic on soybean



Soybean seedling roots with root rot symptoms.

Industry Laboratory. Seedling roots were washed thoroughly before root tissue was tested for Pythium using gene-based methods.

Amplification of DNA from the cytochrome oxidase gene region (Martin 2000, Villa 2006) and sequence analysis allowed for fast and accurate identification to species level without performing time-consuming culturing and morphological observations necessary for classic pathogen identification.

Results - A total of 434 combined root samples were tested from 2011 to 2020. Ninety-five percent (414 of 434) of samples tested positive for *Pythium*. Seventy-nine percent (343 of 434) of samples could be identified to a specific *Pythium* species. Another 16.4% (71 of 434) of DNA sequences could not be determined beyond genus level. Table 2 lists all 23 different *Pythium* species identified during our surveys of Wisconsin soybean fields. It should be noted that recent scientific literature places certain *Pythium* species under the genus name *Globisporangium* (*G. paroecandrum*, *G. ultimum* and *G. sylvaticum*).

DATCP survey findings align with species reported in four recent studies. Research teams in Ohio (Broders et al. 2007), North Dakota (Zitnick-Anderson et al. 2015) and Minnesota (Radmer et al. 2017) investigated *Pythium* diversity and their effect on soybean and corn. An extensive study of oomycetes associated with soybean seedlings in North America identified 51 Pythium species (Rojas et al. 2017). Sixteen of the 23 *Pythium* species detected during our survey caused seed rot and/or seedling root rot in at least one of these studies. DATCP's findings expand the number of species that may have a negative effect on soybean seedling establishment and may ultimately have an impact on yield.

P. sylvaticum, *P. irregulare*, *P. intermedium* and *P. ultimum* are well known and considered most detrimental to soybean seed and seedling roots. *P. sylvaticum* was the most frequently found species in our survey, present in 21.4% of Wisconsin fields. Less often found were *P. irregulare* in 5.1%, *P. intermedium* in 0.2% and *P. ultimum* in 2.5% of fields. *P. torulosum* (3.5%) and *P. conidiophorum* (4.4%) have, more recently, been associated with soybean seedling disease. It is worth noting that all these species are pathogenic on corn seed and seedlings as well.

P. recalcitrans (9.2%) and *P. attrantheridium* (6.5%) are newly described species and as far as we know, had never been reported in Wisconsin soybean fields until our survey in 2012 (Moralejo et al 2008, Allain-Boule et al 2004). *P. recalcitrans* was reported on carrots in Michigan (Lu et al 2013), grape vine roots in South Africa and beets in Spain (Moralejo et al 2008). Seed assays conducted by Minnesota investigators determined that *P. recalcitrans* is pathogenic on both soybean and corn (Radmer et al 2017).

Several *Pythium* found during our soybean survey are historically associated with other hosts: such as *P. arrhenomanes* with corn and *P. heterothallicum* with wheat. Species like *P. aphanidermatum* are very destructive on greenhouse plants and infect a large variety of hosts (Farr and Rossman 2013, Van Der Plaats-Niterink 1981). *P. violae* causes cavity spot on carrots (Schrandt 1994).

Identification of Pythium and its relatives to species level has become more feasible for diagnostic laboratories since gene-based methods have become more readily available and affordable. Although our survey may not account for all species present, it documents a remarkable diversity of Pythium species in Wisconsin soybean fields.

Variability in species, temperature-dependent pathogenicity and multiple species infections can complicate effective control. In fields with a history of seedling disease an integrated management approach may be needed to control disease, including consideration of rotation strategies, given the reported host range of several of these species. For further information please see the University of Wisconsin Field Crops Pathology website, http://fyi.uwex.edu/fieldcroppathology/.

Literature:

Allain-Boule, N., Tweddell, R., Mazzola, M., Belanger R., Levesque, C. A., 2004. Pythium attrantheridium sp. nov.: Taxonomy and Comparison with related species. Mycol. Res. 108 (7): 795-805.

Bala K., Robideau G.P., Lévesque C.A., et al. 2010. Phytopythium Abad, de Cock, Bala, Robideau, Lodhi & Lévesque, gen. nov. and Phytopythium sindhum Lodhi, Shahzad & Lévesque, sp. nov. Fungal Planet 49. Persoonia 24:136-137.

Broders, K. D., Lipps, P. E., Paul, P. A., Dorrance, A. E. 2007. Characterization of Pythium spp. associated with corn and soybean seed and seedling diseases in Ohio. Plant Disease 91:727-735.

Choudhary, C.E., Burgos-Garay, M.L., Moorman, G.W., Hong, C. Pythium and Phytopythium Species in Two Pennsylvania Greenhouse Irrigation Water Tanks. Plant Disease. 2016. May; 100 (5):926-932.

Farr, D.F., Rossman, A.Y. 2013. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA.

Lu, X.H., Jiang, H.H., Hao, J. J. 2013. First Report of *Pythium recalcitrans* causing cavity spot of carrot in Michigan. Plant Disease 97(7): 991.

Martin, F.N. 2000. Phylogenetic relationship among some Pythium species inferred from sequence analysis of the mitochondrially encoded cytochrome oxidase II gene. Mycologia 92 (4):711-727.

Moralejo E., Clemente, A., Descals, E., Belbahri, L., Calmin, G., Lefort, F., Spies, C. F. J., McLeod, A. 2008. Pythium recalcitrans sp. nov. revealed by multigene phylogenetic analysis. Mycologia 100 (2), pp. 310-319.

Radmer, L., Anderson, G., Malvick, D. M., Kurle, J. E., Rendahl, A. and Mallik, A. 2017. Pythium, Phytophthora, and Phytopythium spp. Associated with Soybean in Minnesota, Their Relative Aggressiveness on Soybean and Corn, and Their Sensitivity to Seed Treatment Fungicides. Plant Disease 101: pp. 62-72.

Rojas J. A., Jacobs J.L., Napieralski S., Karaj B., Bradley C.A., Chase T., Esker P.D., Giesler L.J., Jardine D.J., Malvick D.K., Markell S.G., Nelson B.D., Robertson A.E., Rupe J.C., Smith D.L., Sweets L.E., Tenuta A.U., Wise K.A., Chilvers M.I. 2017. Oomycete Species Associated with Soybean Seedlings in North America-Part I: Identification and Pathogenicity Characterization. Phytopathology, Volume 107, Number 3, Pages 280-292

Schrandt, J. K, Davis, R. M. and Nunez, J. J. 1994. Host range and influence of nutrition, temperature, and pH on growth of *Pythium violae* from carrot. Plant Disease 78, 335-338.

Van Der Plaats-Niterink, J. 1981. Studies in Mycology. No 21. Monograph of the Genus Pythium.

Villa N. O., Kageyama K., Asano K, Suga H., 2006. Phylogenetic relationships of Pythium and Phytophthora species based on ITS rDNA, cytochrome oxidase II and b-tubulin gene sequences. Mycologia, 98(3) pp. 410-422.

Zitnick-Anderson, K. K., and Nelson, B. D., Jr. 2015. Identification and pathogenicity of Pythium on soybean in North Dakota. Plant Dis. 99:31-38.

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