

**Four new host and three new state records of *Dothistroma* needle blight caused by  
*Dothistroma pini* in the U.S.A.**

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During 2010 and 2011, *Dothistroma* needle blight (DNB), also known as red band needle blight, was observed for the first time in Cass and Pembina Counties in North Dakota (ND). In Pembina Co., DNB was observed in two sites in the Jay V. Wessles Wildlife Management Area (JWWMA). In September, 2009, yellow spots on green needles were observed on some trees along the western edge of one planting. By June 2010, DNB was found on third- and fourth-year needles in both JWWMA plantings. Symptoms had developed into dark brown bands or spots on necrotic needles that contained erumpent black acervuli. In June 2011, similar DNB symptoms were observed on *P. nigra*, *P. flexilis*, *P. ponderosa*, *P. cembra* and *P. albicaulis* in the Dale E. Herman Research Arboretum, Cass Co., ND. DNB was collected in July 2011 in Brookings Co., South Dakota (SD) from a seed source provenance planting of *P. ponderosa*. To identify the species causing the infections, symptomatic needles were collected in 2010 from both sites in JWWMA and then again from all four locations in 2011 on all pine species infected (Table 1). Needles of *P. nigra* from a private residence near Fairland in Shelby County, Indiana (IN) were also included in the sample set. The rDNA-ITS was PCR-amplified either directly from conidia obtained from acervuli on the needles or from cultures obtained from isolations. Amplicons were sequenced and a blast search was performed in GenBank. The sequences of samples obtained from *P. nigra*, *P. flexilis*, *P. cembra* and *P. albicaulis* in ND, *P. ponderosa* in SD and *P. nigra* from IN showed 100% sequence homology with *D. pini* (Accession No. AY808302). These isolates were identical to all previously assayed isolates of *D. pini* from Nebraska, Minnesota and Michigan in the USA. The *P. ponderosa* isolates from all three sites in ND differed from the other isolates and contained a 1bp point mutation from a C to a T at site 72 (sequence deposited in GenBank with Accession No. KJ933441). Mating type was determined using species-specific mating type primers for *D. pini* (3). All 26 samples from ND and SD were of the MAT-1 idiomorph, while the sample from IN contained the MAT-2 idiomorph (Table 1). All cultures are maintained at FABI, University of Pretoria, South Africa. The two species that cause DNB, *D. septosporum* (G. Dorog.) M. Morelet and *D. pini* Hulbary, are morphologically indistinguishable and molecular characterisation remains essential for correct species identification (1). Host and geographical distribution range determinations of *Dothistroma* species made without molecular methods are not valid. To date, species confirmed using DNA-sequences in the USA include *D. septosporum* in the Pacific Northwest states of Oregon and Idaho on *P. ponderosa*, and

**Table 1.** Detailed information pertaining to the geographic locations and isolate information of *Dothistroma pini* identified in this study.

State	County/State	Site	Date sampled	Longitude	Latitude	Legal description	Host species	CMW number <sup>a</sup>	NDSU accession number <sup>b</sup>	Substrate used for identification	100% ITS sequence homology with GenBank No.	Mating type
North Dakota	Cass County	North Dakota State University Dale E.	29 June 2011	97°21'12"W	46°59'11"N	T140N R53W NE Section35	<i>P. nigra</i>	37624	89208	culture	AY808302	MAT1
			29 June 2011	97°21'12"W	46°59'11"N	T140N R53W NE Section35	<i>P. nigra</i>	37625	89208	culture	AY808302	MAT1
		Herman Research Arboretum	29 June 2011	97°21'12"W	46°59'11"N	T140N R53W NE Section35	<i>P. nigra</i>	37626	89208	culture	AY808302	MAT1
			29 June 2011	97°21'12"W	46°59'11"N	T140N R53W NE Section35	<i>P. nigra</i>	37627	891	culture	AY808302	MAT1
		29 June 2011	97°21'12"W	46°59'11"N	T140N R53W NE Section35	<i>P. nigra</i>	37628	891	culture	AY808302	MAT1	
		29 June 2011	97°21'12"W	46°59'11"N	T140N R53W NE Section35	<i>P. nigra</i>	37629	891	culture	AY808302	MAT1	
		29 June 2011	97°21'12"W	46°59'11"N	T140N R53W NE Section35	<i>P. nigra</i>	37630	82367	culture	AY808302	MAT1	
		29 June 2011	97°21'12"W	46°59'11"N	T140N R53W NE Section35	<i>P. nigra</i>	37631	82367	culture	AY808302	MAT1	
		29 June 2011	97°21'12"W	46°59'11"N	T140N R53W NE Section35	<i>P. flexilis</i>	-	84264	acervuli	AY808302	MAT1	
		29 June 2011	97°21'12"W	46°59'11"N	T140N R53W NE Section35	<i>P. ponderosa</i>	37632	75299	culture	KJ933441	MAT1	
		29 June 2011	97°21'12"W	46°59'11"N	T140N R53W NE Section35	<i>P. ponderosa</i>	37633	75299	culture	KJ933441 <sup>c</sup>	MAT1	
		06 July 2011	97°21'12"W	46°59'11"N	T140N R53W NE Section35	<i>P. cembra</i>	-	88118	acervuli	AY808302	MAT1	
		06 July 2011	97°21'12"W	46°59'11"N	T140N R53W NE Section35	<i>P. cembra</i>	37634	7724	culture	AY808302	MAT1	
		06 July 2011	97°21'12"W	46°59'11"N	T140N R53W NE Section35	<i>P. flexilis</i>	-	79267	acervuli	AY808302	MAT1	
	06 July 2011	97°21'12"W	46°59'11"N	T140N R53W NE Section35	<i>P. albicaulis</i>	-	7728	acervuli	AY808302	MAT1		
	Pembina County, Jay V. Wessels Wildlife Management Area (JWWMA)	Wildlife habitat planting =/- 15 years old	17 June 2010	97°52'55"W	48°49'7"N	T162N R56W NW Section 34	<i>P. ponderosa</i>	41115	-	culture	KJ933441	MAT1
			17 June 2010	97°52'55"W	48°49'7"N	T162N R56W NW Section 34	<i>P. ponderosa</i>	41116	-	culture	KJ933441	MAT1
		Wildlife Management Area (JWWMA)	17 June 2010	97°52'55"W	48°49'7"N	T162N R56W NW Section 34	<i>P. ponderosa</i>	41117	-	culture	KJ933441	MAT1
			30 July 2011	97°52'55"W	48°49'7"N	T162N R56W NW Section 34	<i>P. ponderosa</i>	37605	-	culture	KJ933441	MAT1
		30 July 2011	97°52'55"W	48°49'7"N	T162N R56W NW Section 34	<i>P. ponderosa</i>	37612	-	culture	KJ933441	MAT1	
30 July 2011		97°52'55"W	48°49'7"N	T162N R56W NW Section 34	<i>P. ponderosa</i>	37616	-	culture	KJ933441	MAT1		
Seed source provenance planting, 22 yrs old, planted 1988		17 June 2010	97°50'34"W	48°48'37"N	T162N R56W SE Section 35	<i>P. ponderosa</i>	41118	-	culture	KJ933441	MAT1	
		17 June 2010	97°50'34"W	48°48'37"N	T162N R56W SE Section 35	<i>P. ponderosa</i>	41119	-	culture	KJ933441	MAT1	
South Dakota	Brookings County	Seed source provenance planting, planted 1988	15 July 2011	96°44'26"W	44°19'25"N	T106N R19W NW Section 20	<i>P. ponderosa</i>	38037	-	culture	AY808302	MAT1
			15 July 2011	96°44'26"W	44°19'25"N	T106N R19W NW Section 20	<i>P. ponderosa</i>	37919	-	culture	AY808302	MAT1
			15 July 2011	96°44'26"W	44°19'25"N	T106N R19W NW Section 20	<i>P. ponderosa</i>	37963	-	culture	AY808302	MAT1
Indiana	Shelby County	Ornamental planting at residence near Fairland	20 May 2011	85°51'39" W	39°38'27" N	T14N R6E SE Section 21	<i>P. nigra</i>	37786	-	culture	AY808302	MAT2

<sup>a</sup> Culture collection of the Forestry and Agricultural Biotechnology Institute, FABI, South Africa.<sup>b</sup> Accession number for the herbarium collection at the North Dakota State University Dale E Herman Research Arboretum.<sup>c</sup> ITS sequence deposited into GenBank in this study.

*D. pini* in the North Central states of Nebraska, Minnesota and Michigan on *P. nigra* (1). This study documents the presence of *D. pini* in three additional USA states, including a first report of DNB in ND and SD. It also includes new records of *D. pini* infecting *P. flexilis*, *P. cembra*, *P. albicaulis* and *P. ponderosa*. Results of this study have expanded the documented host range of *D. pini* in the USA from one (*P. nigra*) to five species. Globally, *D. pini* is now known to infect a total of 10 pine hosts (2, 4, 5).

*References:*

- (1) I. Barnes et al. Stud. Mycol. 50:551, 2004.
- (2) I. Barnes et al. For. Pathol. 41:361, 2011.
- (3) M. Groenewald et al. Phytopathology 97:825, 2007.
- (4) D. Piou et al. Plant Disease. 98:841, 2014.
- (5) B. Piskur et al. For. Pathol. 43:518, 2013.