

# Diplodia Tip Blight of Ponderosa Pine in the Black Hills of South Dakota

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## ABSTRACT

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*Diplodia* tip blight was found for the first time on native ponderosa pine at scattered locations in the Black Hills of South Dakota. Infection of new shoots occurred annually in 1979-1982; however, extensive infection of second-year needle and branch tissues occurred only in 1979 after an unusually cold winter. Black Hills isolates of *Diplodia pinea* did not differ significantly from isolates from other areas in spore dimensions, spore septations, spore germination, germ tube growth, or growth in culture.

*Diplodia* tip blight, incited by *Diplodia pinea* (Desm.) Kickx. (*Sphaeropsis sapinea* (Fr.) Dyko & Sutton), was viewed by the first author on ponderosa pine (*Pinus ponderosa* Laws.) in the Black Hills of South Dakota in July 1979. This was the first known case of infection in native stands of ponderosa pine. Reports of the disease from around the world concern infection of pines in plantations or shelterbelts. The fungus causes death of current-year shoots and branches and sometimes entire trees (5). New shoots become infected early in the growing season during a period of high susceptibility that begins when buds expand and ends when shoot elongation ceases (1,3). Commonly, all tissues of infected new shoots are killed. Under some conditions, infection spreads into branches and needle tissues that formed the previous year. Damage may then be extensive and highly visible.

Surveys were conducted during 1979-1982 to determine the distribution of the disease throughout the Black Hills and to follow the progress of disease symptoms. Cultural and spore characteristics of isolates of *D. pinea* from the Black Hills were compared with isolates from other areas to determine if there were major differences. The Black Hills isolates were also tested to determine if they were pathogenic to ponderosa pine.

## MATERIALS AND METHODS

Field examinations of symptomatic ponderosa pines were conducted annually

along major highways throughout the Black Hills during June, July, or August in 1979-1982. Stops were made to confirm the identity of the disease. Necrotic tissues including cone scales, twigs, needles, and dead buds from symptomatic trees were examined for pycnidia. Observations were made to determine if infection was confined to new shoots or whether second-year branch and needle tissues also were infected. In addition, second-year cones were collected in 10 widely separated areas where trees were free of shoot infection. These cones were examined for pycnidia of *D. pinea*.

Observation plots were established in two areas (Pennington County and Rockerville) to follow the progress of symptoms for several seasons (Fig. 1). Data recorded for trees included diameter at breast height, age, cone abundance, and disease rating, which was a modification of that used for dwarf mistletoe (2). Individual tree crowns, viewed from the ground, were divided visually into thirds and each third was rated as 0 = not infected, 1 = lightly infected, or 2 = heavily infected, on the basis of the presence of dying twigs and branches. Disease ratings were recorded in 1980, 1981, and 1982 and compared.

Weather data (monthly high and low temperatures and precipitation) collected about 29 km northwest of Rapid City, SD, from 1975 to 1980 were examined for trends or events related to the sudden appearance of the disease in 1979.

Tissue samples, including needles, buds, and cones, were collected from symptomatic trees throughout the Black Hills. Samples that on initial examination did not have pycnidia were incubated at 100% relative humidity and 24 C for 48 hr, then reexamined. Identification was based on the presence of both pycnidia and spores typical of *D. pinea* (6).

To test pathogenicity of Black Hills

isolates, suspensions of spores obtained from cultures of the fungus were brushed on ponderosa pine seedlings. Inoculated seedlings were then kept under continuous light ( $323 \mu\text{W}/\text{cm}^2$ ) in growth chambers at 24 C and 100% relative humidity for 48 hr, then moved to a greenhouse (night temperature 24 C). After symptoms developed (4-8 days), needle tissue was surface-sterilized (50% ethanol for 1 min) and incubated on potato-dextrose agar (PDA) at 24 C in the light. Resulting colonies were examined for pycnidia and spores typical of *D. pinea*.

Dimensions of spores of 27 isolates of *D. pinea*, including nine from the Black Hills, were measured. Spores were obtained from pycnidia produced in PDA cultures of the isolates that had been incubated in the light ( $92 \mu\text{W}/\text{cm}^2$ ) at 28 C. For each isolate, 90 spores (30 from each of three pycnidia) were measured with an ocular micrometer at

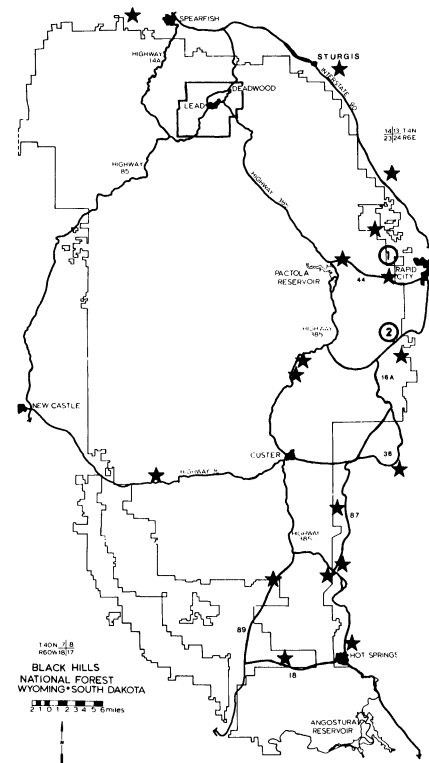


Fig. 1. Locations (stars) where *Diplodia pinea* was confirmed by isolation from infected ponderosa pine in the Black Hills of South Dakota (1979-1982). Circled numbers indicate observation plots: 1 = Pennington County and 2 = Rockerville. Bold lines are highways along which the survey was conducted.

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×430. The number of septate spores in each group was also recorded.

Spores for germination tests were from pycnidia produced in cultures as described previously. Spores were placed on 2% water agar in petri dishes by dipping a glass rod (8 mm in diameter) into a spore suspension, then touching the agar surface. Spores were incubated at 16, 20, 24, 28, or 32 C (±0.5 C) for 4 hr in dark incubators, then killed with mercuric chloride. Germ tubes (30) were measured with an ocular micrometer, and germinated spores (no. in 100) were counted at ×430 in each of three replicates.

Mycelial growth of Black Hills isolates was compared with that of isolates from Nebraska, Oklahoma, and Chile. Included in the growth test were isolates from *P. ponderosa*, *P. nigra* Arnold, *P. sylvestris* L., *P. mugo* Turra, and *P. radiata* D. Don; mass-spore and single-spore isolates; and isolates from both needles and second-year seed cones. Dishes were seeded in the center with 4-mm<sup>2</sup> disks from margins of 2-day-old cultures. Cultures were incubated in the light (92 μW/cm<sup>2</sup>) for 48 hr at 28 C, then colony diameters were measured. The test was run in triplicate.

The Statistical Analysis System (SAS) General Linear Models Procedure was used to analyze data (spore dimensions, colony diameters, germ tube lengths, and germination). Comparisons (*P* = 0.05) were made between regions (Black Hills vs. other areas) and among areas (Custer, SD, eastern Nebraska, northwestern Nebraska, Chile, and Oklahoma) within regions.

## RESULTS AND DISCUSSION

*D. pinea* was confirmed in several locations scattered primarily along the eastern half of the Black Hills from Sturgis to Hot Springs (Fig. 1). The scattered occurrence suggested the fungus was not recently introduced. The disease did not appear to be increasing in severity on trees observed over a 3-yr period or to be spreading into new areas in the Black Hills. In fact, incidence and severity declined after 1979.

The best indication of *D. pinea* was the presence of pycnidia on scales of second-year and older seed cones. The disease appeared most damaging on trees with numerous cones (Table 1) that were located in openings and along the edges of stands. Severely infected trees had numerous dead branches. Tree mortality was uncommon. The fungus was not found on cones in areas where trees were free of shoot infection.

Severity of infection varied greatly (from a rating of 0 to 6) on trees in the

**Table 1.** Description and disease ratings of ponderosa pines in plots in the Black Hills of South Dakota

Plot location	Tree no.	Tree age (yr)	Tree dbh (cm)	Cone abundance <sup>a</sup>	Disease ratings <sup>b</sup>		
					1980	1981	1982
Pennington County	1	85	59.2	H	6	5	6
	2	75	43.2	L	4	3	3
	3	72	35.6	L	3	3	3
	4	80	58.9	H	6	5	6
	5	80	67.3	N	0	0	2
	6	69	40.1	N	0	0	0
	7	110	51.0	H	6	6	6
Rockerville	1	74	51.3	H	6	5	3
	2	63	41.1	M	5	2	3
	3	75	44.2	N	0	0	2
	4	78	34.3	L	2	2	1
Average		78.3	47.7		3.4	2.8	3.2

<sup>a</sup> H = heavy, M = medium, L = light, and N = none.

<sup>b</sup> Each datum is the sum of ratings of each third of the tree crown, where 0 = not infected, 1 = lightly infected, and 2 = heavily infected.

plots and on most trees did not change greatly from year to year (Table 1).

Infection was not detected in managed, fully stocked stands, thus changes in current silvicultural practices are not necessary at this time. Trees of high value in developed recreation sites and around homesites can be protected by chemical sprays if warranted (4,5).

The development of disease in the Black Hills did not differ appreciably from that observed by Peterson (3) in plantations in eastern Nebraska, except infection of expanding buds occurred with much greater frequency in the Black Hills than in eastern Nebraska. This may be related to the later development of buds in the Black Hills. In eastern Nebraska, new needles are just breaking through fascicle sheaths in mid-May in most years; this stage of development was not reached in the Black Hills until the first week of June in 1980.

In both areas, new shoots are infected early and these are usually killed. Also in both areas, infection in some years is not confined to new shoots but extends to second-year needle and stem tissues. The extensive infection in 1979 was not related to known insect activity. Possibly, the stress caused by high incidence of infected new shoots produced the conditions favorable for infection of second-year tissues. Also, cold weather preceding the growing season may have predisposed the second-year tissues to colonization. The winter of 1978–1979 in the Black Hills was unusually cold. A predisposing role of cold weather is suggested by observations in eastern Nebraska. Infection of both first- and second-year tissues in 1982 was the most severe in eastern Nebraska in 25 yr (G. W. Peterson, unpublished) after one of the coldest winters on record.

All nine Black Hills isolates were pathogenic to ponderosa pine seedlings. On morphological and cultural bases, the Black Hills isolates were not significantly different (*P* = 0.05) from the isolates from other regions (Nebraska, Oklahoma, and Chile).

The average length and width of spores was 28.5 × 12.5 μm for the Black Hills isolates and 29.4 × 12.7 μm for the other isolates. The percentage of septate spores of the Black Hills isolates averaged 5.5% and the other isolates averaged 6.3%. Colony diameters of isolates incubated at 28 C on PDA for 48 hr averaged 70.5 mm (Black Hills isolates) and 70.8 mm (other isolates). The percentage of germination of spores of all isolates over the range of temperatures (16–32 C) averaged 93.2%. The average percentage of germination was similar for both the Black Hills isolates and the other isolates. Growth of germ tubes of all Black Hills isolates and all other isolates was optimum at 28 C.

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