

Resistance to *Dothistroma pini* Within Geographic Seed Sources of *Pinus ponderosa*

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## ABSTRACT

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In plantings of *Pinus ponderosa* established in 1968 in Nebraska, Oklahoma, and Missouri, seedlings from a total of 78 geographic seed sources were tested for resistance to *Dothistroma pini* which causes needle blight. There was high resistance to *D. pini* in seedlings from six of the sources: four from New Mexico, one from Arizona, and one from

Nebraska. Trees from most of these sources were above plantation averages in height after 10 yr of growth. Thus, there are a number of areas where seed can be collected for the production of seedlings that may be planted where *D. pini* is a threat.

*Additional key words:* *Scirrhia pini*.

Investigations of the biology of needle blight caused in pines by *Dothistroma pini* Hulbary have led to the development of effective methods for direct control of this fungus by applying chemicals (4-6, 8-10). Studies of resistance to *D. pini* have been limited (1,2,16); however, from general observations and from the results of some studies there is evidence that the impact of the disease could be further reduced by use of genetic resistance. Seed from Yugoslavia is currently used to produce Austrian pine (*Pinus nigra* Arnold) for Great Plains plantings; a study in eastern Nebraska showed that trees grown from this seed were highly resistant to *D. pini* and also grew well (11).

A comprehensive study (12) of *Pinus ponderosa* Dougl. ex Laws. was initiated to identify geographic sources of seed whose use would result in improved plantings of ponderosa pine in the Great Plains. Seventeen plantations, each containing seedlings from 79 or fewer geographic seed sources of *P. ponderosa*, were established in spring 1968 to provide data on survival and growth. The plantings have also provided an opportunity to investigate resistance to *D. pini* among the geographic seed sources (7).

## MATERIALS AND METHODS

**Host trees.** Seed collections were made at locations where the climate most closely resembled that of the Great Plains region. Most of the natural ponderosa pine stands east of the Continental Divide were sampled (Fig. 1). Locations and climatic and sampling data for the collections are contained in Read (12). Seven to 18 trees were selected from each of the stands. The intent was not to select the best trees, but instead to obtain a representative sample of the wide range of phenotypes. Seeds from each stand were composited (12).

Three of the plantings were used for disease resistance investigations; they are located in Nebraska, Missouri, and Oklahoma. The Nebraska planting was established primarily for the study of needle blight caused by *D. pini*; the fungus was artificially introduced into this planting. *Dothistroma* blight epidemics developed in the Missouri and Oklahoma plantings without artificially introducing *D. pini*. All plantations were established with 3-yr-old seedlings.

The Nebraska planting was established on the University of Nebraska's Horning State Farm, near Plattsmouth, in eastern

Nebraska. The experimental layout consists of 10-tree linear plots representing 50 of the 79 seed sources, with six replicates in a randomized block design. Trees are spaced 2.4 m apart in rows 4 m apart. The plantation site is at 41.0 north latitude, 95.9 west longitude, elevation 335 m, and is on a west-facing slope of deep silt loam soil derived from loess. The growing season averages 170 days, and the mean annual precipitation is 762 mm. Weeds were controlled from 1968 through 1972 by mowing between rows and by applying herbicide along both sides of the tree rows in early spring. Maintenance since 1972 has involved only periodic mowing. The trees were sprayed with dimethoate in the early summer of 1976 and 1977 to reduce the effects of a tip moth (*Rhyacionia bushnelli* Busck) infestation on disease evaluations.

*Dothistroma pini* was introduced into the Nebraska planting on infected needles of *Pinus nigra* growing 1 km from the ponderosa pine planting: In early summer, bundles containing 8 to 10 infected needles were attached with string to the previous year's shoots. Inoculations were made in successive years, 1971-1975, to disperse *D. pini* in the planting. By spring 1975, the fungus was well distributed throughout the planting. However, rainfall was abnormally low during the 1975 and 1976 growing seasons; and although infection was widespread, infection levels were too low to evaluate resistance. Additional inoculations were made in 1978. Moisture was very favorable for infection in 1978, 1979, 1980, and 1981. Because the disease was epidemic during 1979-1981, each tree in the planting was evaluated during those years.

The Oklahoma planting is at the Oklahoma State Nursery, which is in the center of the state, near Norman. The experimental layout consists of four-tree linear plots representing 40 seed sources, with 15 replicates in a randomized block design. Trees are spaced at 3.6 m in rows 3.6 m apart. The plantation site is at 35.2 north latitude, 97.5 west longitude, elevation 335 m, and is on a level plain of deep sandy loam soil. The growing season averages 213 days, and the mean annual precipitation is 743 mm. The planting was cultivated annually until 1973 to reduce weed competition.

The Missouri planting is on the University of Missouri's South Farm near Columbia. The planting includes trees from 78 sources in a completely randomized design, with eight replicates of four-tree linear plots. Trees are spaced at 2.4 m apart in rows 2.4 m apart. The plantation site is at 38.9 north latitude, 92.4 west longitude, elevation 213 m, and is on a level plain of silt loam surface soil overlying a clay pan. The growing season averages 189 days, and the mean annual precipitation is 960 mm.

**Disease evaluations.** To evaluate resistance of the sources, each tree was examined and a separate infection rating was given to both first-year (current-year) and second-year needles based on presence of and degree of *Dothistroma* blight symptoms. This was done because ponderosa pine needles of different ages are not equally

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susceptible to *D. pini* (5). Ratings were made after symptoms were fully developed, usually in late fall or in early winter. Fortunately, trees in the plantings had not been infected by other foliar pathogens (ie, *Scirrhia acicola*) causing symptoms that might be confused with those caused by *D. pini*. All evaluations were made by the same two people. Trees were observed from both sides of tree rows, because infection is frequently more severe on the north-northeast sides of trees. The following rating scheme was used: 1 = 0 to 3 lesions per needle; 2 = 4 to 10 lesions; and 3 = >10 lesions per needle. The rating of a given age class of needles on a tree was not based on averaging of numbers of lesions on a specific number of needles on a specific number of shoots; rather observations of needles were made over the whole tree and ratings made based on numbers of lesions on needles of the most severely infected shoots. Evaluations were made in Missouri in 1980, in Nebraska in 1979, 1980, and 1981, and in Oklahoma in 1979 and 1980. The data presented in this paper are from the 1980 evaluation in Missouri, the 1981 data in Nebraska, and the 1979 data in Oklahoma. In the Oklahoma planting, there was less infection in 1980 because of a severe drought; there was more infection in the Nebraska planting in 1981 than in either 1980 or 1979.

Initially, the statistical significance of differences in resistance of second-year needles among sources was examined by using analysis of variance ( $\alpha = 0.05$ ). Subsequent application of Duncan's multiple range test produced a characteristic result when applied to a large number of sources. Sources with extreme differences in resistance were identified, but sources with intermediate resistance were grouped with a multilayered series of overlapping designations, thereby making evaluation of geographic pattern in resistance difficult. Toward a goal of a more interpretable analysis, the source means were partitioned into groups with similar

resistance by a cluster analysis method (15). Unlike a multiple range test which would identify pairwise differences in resistance among sources, the cluster analysis method identifies group centers in a way that maximizes between-group variation (or equivalently, minimizes within-group variation). Similar to analysis of variance, significance of a particular partition of the sources is assessed by comparing between-group versus within-group estimates of variance with an adjustment of the latter to incorporate the residual variance estimated in the initial analysis of variance. The data are clustered into successively larger numbers of groups until between- and within-group estimates of variance are not significantly different ( $\alpha = 0.05$ ). The result is a nonsubjective partitioning of the sources according to their resistance, which can be evaluated for geographic pattern. This analysis provides no information about differences between individual sources, but it does assess the significance of differences between the centers of groups of sources.

## RESULTS

Graphical evaluation of standardized residuals from the analysis of variance indicated little departure from normality for any of the three plantations. However, heterogeneous variance was indicated for the Missouri and Oklahoma plantations. Because analysis of variance with equal replication is robust to heterogeneous variance but multiple range tests are not (14), the use of the overall *F*-test from the analysis of variance of each plantation was considered justified. The presence of heterogeneous variance indicated that the multiple range test results should be discarded whether or not they are readily interpretable. The cluster analysis also probably is adversely affected by heterogeneous variance but, because it employs a significance criterion similar to the *F*-test in the analysis

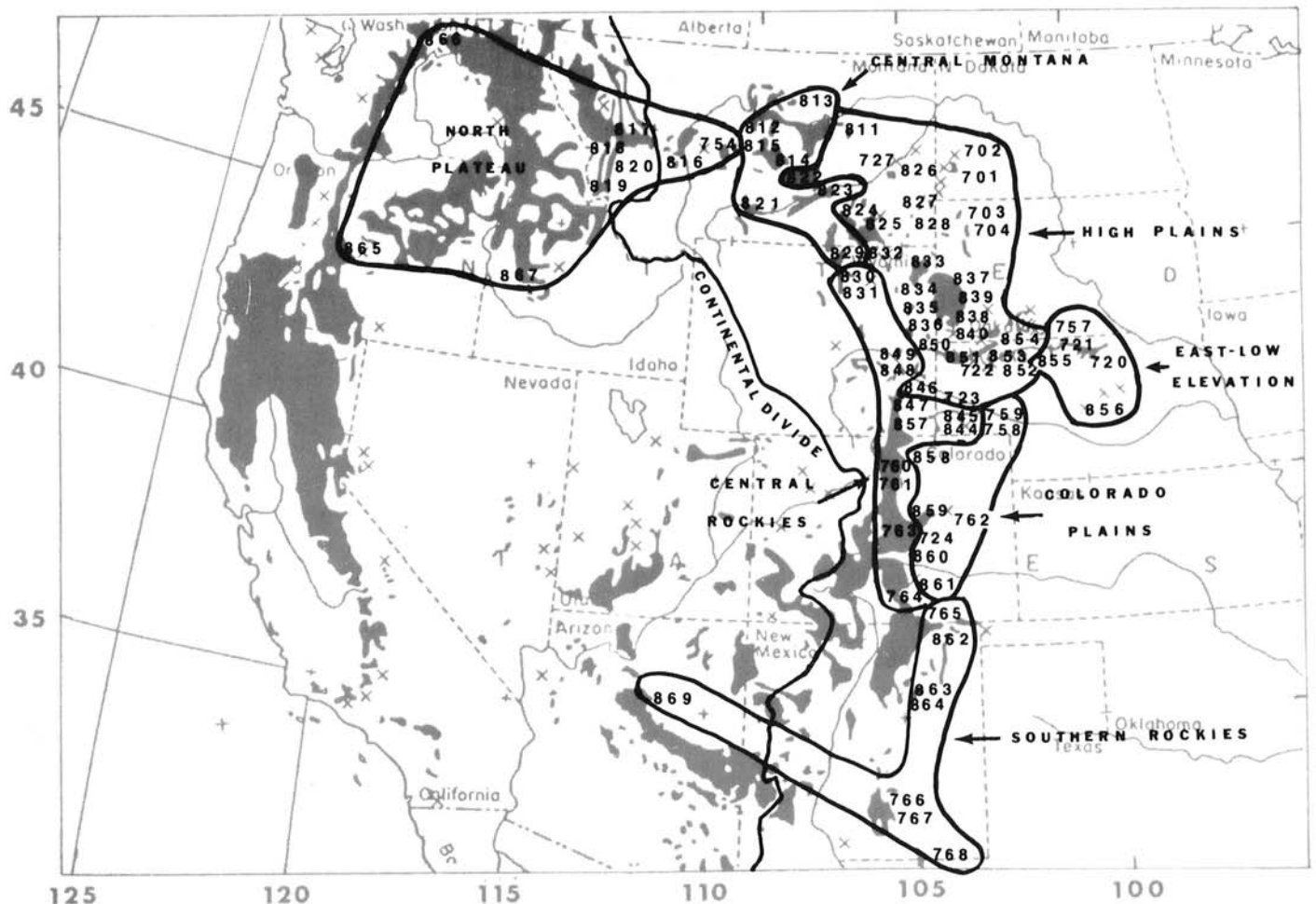


Fig. 1. Location of geographic seed sources of ponderosa pine. Shaded areas indicate natural distribution of ponderosa pine (3). Geographic delineations (clusters) are from Read (12).

TABLE 1. Infection of geographic seed sources of *Pinus ponderosa* by *Dothistroma pini* in three plantations

| Geographic seed <sup>w</sup><br>source |          | Infection rating <sup>x</sup> in plantations in: |                   |                                |                   |                                |                   |
|--|----------|--|-------------------|--------------------------------|-------------------|--------------------------------|-------------------|
|  |          | Missouri <sup>y</sup>                            |                   | Nebraska <sup>y</sup>          |                   | Oklahoma <sup>y</sup>          |                   |
| Number                                 | Location | 2nd-yr<br>needles <sup>z</sup>                   | 1st-yr<br>needles | 2nd-yr<br>needles <sup>z</sup> | 1st-yr<br>needles | 2nd-yr<br>needles <sup>z</sup> | 1st-yr<br>needles |
| 869                                    | AZ       | 1.22 a   | 1.0               | 1.33 a                         | 1.0               | 1.00 a                         | 1.1               |
| 767                                    | NM       | 1.39 a   | 1.0               | 1.18 a                         | 1.0               | 1.09 a                         | 1.0               |
| 768                                    | NM       | 1.41 a   | 1.0               | 1.43 a                         | 1.0               | 1.14 a                         | 1.0               |
| 864                                    | NM       | 1.54 a   | 1.0               | 1.65 a                         | 1.0               | 1.13 a                         | 1.0               |
| 766                                    | NM       | 1.54 a   | 1.0               | 1.27 a                         | 1.0               | 1.14 a                         | 1.0               |
| 720                                    | NE       | 1.60 a   | 1.0               | 1.17 a                         | 1.0               | 1.11 a                         | 1.0               |
| 853                                    | NE       | 1.72 b   | 1.0               | 2.07 b                         | 1.2               | ...                            | ...               |
| 854                                    | SD       | 1.80 b   | 1.0               | 1.77 b                         | 1.1               | ...                            | ...               |
| 838                                    | SD       | 1.80 b   | 1.0               | 1.57 a                         | 1.0               | ...                            | ...               |
| 765                                    | CO       | 1.81 b   | 1.0               | 1.80 b                         | 1.1               | 1.43 b                         | 1.1               |
| 855                                    | NE       | 1.82 b   | 1.0               | ...                            | ...               | 1.47 b                         | 1.1               |
| 863                                    | NM       | 1.86 b   | 1.2               | 1.35 a                         | 1.0               | 1.12 a                         | 1.0               |
| 827                                    | MT       | 1.89 b   | 1.0               | 1.52 a                         | 1.1               | ...                            | ...               |
| 824                                    | MT       | 1.90 b   | 1.0               | 2.02 b                         | 1.2               | ...                            | ...               |
| 757                                    | SD       | 1.90 b   | 1.0               | ...                            | ...               | ...                            | ...               |
| 727                                    | MT       | 1.94 b   | 1.0               | ...                            | ...               | ...                            | ...               |
| 721                                    | NE       | 1.95 b   | 1.0               | 1.53 a                         | 1.0               | 1.37 b                         | 1.2               |
| 833                                    | WY       | 1.96 b   | 1.0               | 2.08 b                         | 1.3               | ...                            | ...               |
| 862                                    | NM       | 2.01 b   | 1.0               | 1.80 b                         | 1.0               | 1.31 b                         | 1.0               |
| 846                                    | WY       | 2.09 c   | 1.0               | 2.32 b                         | 1.6               | ...                            | ...               |
| 828                                    | MT       | 2.12 c   | 1.0               | ...                            | ...               | ...                            | ...               |
| 856                                    | NE       | 2.14 c   | 1.0               | 1.80 b                         | 1.0               | 1.23 b                         | 1.0               |
| 839                                    | SD       | 2.19 c   | 1.1               | ...                            | ...               | ...                            | ...               |
| 703                                    | SD       | 2.19 c   | 1.0               | ...                            | ...               | ...                            | ...               |
| 852                                    | NE       | 2.21 c   | 1.0               | 2.17 b                         | 1.2               | 1.99 d                         | 1.5               |
| 821                                    | MT       | 2.22 c   | 1.0               | ...                            | ...               | 1.99 d                         | 1.6               |
| 834                                    | WY       | 2.24 c   | 1.0               | 1.78 b                         | 1.1               | ...                            | ...               |
| 762                                    | CO       | 2.26 c   | 1.0               | 2.30 b                         | 1.4               | 1.79 c                         | 1.3               |
| 722                                    | NE       | 2.26 c   | 1.0               | ...                            | ...               | 1.83 c                         | 1.3               |
| 704                                    | SD       | 2.29 c   | 1.0               | 1.57 a                         | 1.1               | 1.58 b                         | 1.3               |
| 822                                    | MT       | 2.30 c   | 1.0               | 2.20 b                         | 1.4               | ...                            | ...               |
| 811                                    | MT       | 2.30 c   | 1.0               | 2.20 b                         | 1.5               | 2.29 d                         | 1.8               |
| 840                                    | SD       | 2.31 c   | 1.0               | 1.73 a                         | 1.2               | 1.91 c                         | 1.3               |
| 825                                    | MT       | 2.31 c   | 1.0               | ...                            | ...               | 2.27 d                         | 1.8               |
| 826                                    | MT       | 2.32 c   | 1.0               | 1.57 a                         | 1.1               | 2.09 d                         | 1.6               |
| 758                                    | NE       | 2.33 c   | 1.0               | 2.60 c                         | 1.5               | 2.06 d                         | 1.6               |
| 861                                    | CO       | 2.34 c   | 1.0               | 2.18 b                         | 1.1               | 1.72 c                         | 1.2               |
| 860                                    | CO       | 2.34 c   | 1.0               | 2.25 b                         | 1.2               | 2.08 d                         | 1.4               |
| 850                                    | WY       | 2.35 c   | 1.0               | ...                            | ...               | ...                            | ...               |
| 823                                    | MT       | 2.36 c   | 1.0               | ...                            | ...               | ...                            | ...               |
| 724                                    | CO       | 2.36 c   | 1.1               | ...                            | ...               | 1.76 c                         | 1.3               |
| 859                                    | CO       | 2.37 c   | 1.0               | 1.68 a                         | 1.2               | ...                            | ...               |

(continued on next page)

of variance, probably much less so than are the multiple range tests. Also, the intent of this analysis was only to evaluate the possibility of geographic patterns in resistance among the seed sources, not to assess differences between individual sources.

For each plantation, analysis of variance indicated significant differences in infection among seed sources ( $P < 0.001$ ). The cluster analysis method divided the seed sources into four subgroups in the Missouri plantation, three in the Nebraska plantation, and five in the Oklahoma plantation. These subgroups are given in Table 1 by the letter a, b, c, d, or e following the infection rating. The first group will be referred to as the "highly resistant" group and contains the sources that were most resistant to *Dothistroma pini*. The last group will be referred to as the "highly susceptible" group and contains the sources that were least resistant.

Six sources were found to be in the highly resistant group for all three plantings. Five of these sources (766, 767, 768, 864, and 869) were from the Southern Rockies (New Mexico and Arizona) and one was from Nebraska (720). All of the "highly resistant" sources had very low levels of infection of first-year needles. No sources had all trees free of infection although some trees within several sources were free of infection. The level of infection of second-year needles was higher or the same as the level of infection of first-year needles in all sources.

Within most of the "highly resistant" sources, there were some trees with highly susceptible second-year needles. For instance, of

the six highly resistant sources 11% (range, 0–20%) of the trees had highly susceptible second-year needles.

Moderate resistance was expressed in all three plantings by several sources (721, 727, 757, 765, 824, 827, 833, 838, 853, 854, 855, 862, and 863). All of these sources had low levels of infection of first-year needles.

The sources in the "highly susceptible" group (754, 760, 761, 813, 816, 817, 818, 820, 829, 831, 857, 865, 866, and 867) were generally from the Central Rockies and the North Plateau. Many of the sources in this group had high levels of infection not only of second-year needles but also of first-year needles. The average infection rating of second-year needles of those sources present in all three plantings was: 2.2 Missouri; 1.9 Nebraska, and 1.7 Oklahoma.

## DISCUSSION

Read (12) delineated geographic patterns of the 78 sources by cluster analysis of seedling characteristics (Fig. 1). The relationships of the geographic groups of sources delineated in his study with the geographic pattern of highly resistant, moderately resistant, and highly susceptible sources are of interest because some of the groups (clusters) delineate varieties and races of *P. ponderosa*. Nine of the 78 sources were of the variety *ponderosa* (12); these nine were all in the "North Plateau" cluster, and eight of

TABLE 1 (continued). Infection of geographic seed sources of *Pinus ponderosa* by *Dothistroma pini* in three plantations

| Geographic seed <sup>w</sup><br>source |          | Infection rating <sup>x</sup> in plantations in: |                   |                                |                   |                                |                   |
|--|----------|--|-------------------|--------------------------------|-------------------|--------------------------------|-------------------|
|  |          | Missouri <sup>y</sup>                            |                   | Nebraska <sup>y</sup>          |                   | Oklahoma <sup>y</sup>          |                   |
| Number                                 | Location | 2nd-yr<br>needles <sup>z</sup>                   | 1st-yr<br>needles | 2nd-yr<br>needles <sup>z</sup> | 1st-yr<br>needles | 2nd-yr<br>needles <sup>z</sup> | 1st-yr<br>needles |
| 835                                    | WY       | 2.38 c   | 1.0               | 2.00 b                         | 1.2               | 1.63 b                         | 1.2               |
| 815                                    | MT       | 2.40 c   | 1.0               | 2.33 b                         | 1.4               | ...                            | ...               |
| 763                                    | CO       | 2.40 c   | 1.0               | 2.17 b                         | 1.4               | ...                            | ...               |
| 858                                    | CO       | 2.42 c   | 1.2               | ...                            | ...               | 1.58 b                         | 1.2               |
| 702                                    | ND       | 2.42 c   | 1.0               | 1.85 b                         | 1.2               | 1.91 c                         | 1.5               |
| 701                                    | ND       | 2.44 c   | 1.0               | ...                            | ...               | ...                            | ...               |
| 836                                    | WY       | 2.45 c   | 1.0               | ...                            | ...               | ...                            | ...               |
| 851                                    | NE       | 2.46 c   | 1.0               | 2.05 b                         | 1.3               | ...                            | ...               |
| 845                                    | NE       | 2.47 c   | 1.0               | 2.30 b                         | 1.4               | ...                            | ...               |
| 837                                    | SD       | 2.51 c   | 1.0               | 1.57 a                         | 1.2               | 1.89 c                         | 1.5               |
| 832                                    | WY       | 2.55 d   | 1.3               | 1.87 b                         | 1.2               | 2.03 d                         | 1.5               |
| 844                                    | NE       | 2.58 d   | 1.0               | ...                            | ...               | ...                            | ...               |
| 759                                    | NE       | 2.58 d   | 1.1               | ...                            | ...               | ...                            | ...               |
| 723                                    | NE       | 2.61 d   | 1.0               | 2.38 b                         | 1.4               | 2.17 d                         | 1.6               |
| 830                                    | WY       | 2.64 d   | 1.1               | ...                            | ...               | 2.27 d                         | 1.7               |
| 812                                    | MT       | 2.64 d   | 1.3               | ...                            | ...               | ...                            | ...               |
| 819                                    | MT       | 2.68 d   | 2.0               | 2.87 c                         | 2.6               | ...                            | ...               |
| 764                                    | CO       | 2.70 d   | 1.0               | 1.83 b                         | 1.2               | 1.41 b                         | 1.1               |
| 847                                    | WY       | 2.74 d   | 1.1               | ...                            | ...               | ...                            | ...               |
| 849                                    | WY       | 2.75 d   | 1.0               | 1.87 b                         | 1.4               | 2.03 d                         | 1.6               |
| 814                                    | MT       | 2.77 d   | 1.0               | ...                            | ...               | ...                            | ...               |
| 848                                    | WY       | 2.79 d   | 1.1               | 2.38 b                         | 1.4               | ...                            | ...               |
| 816                                    | MT       | 2.80 d   | 1.8               | 2.62 c                         | 2.3               | 2.49 e                         | 2.3               |
| 754                                    | MT       | 2.82 d   | 1.6               | ...                            | ...               | 2.53 e                         | 2.2               |
| 813                                    | MT       | 2.85 d   | 1.1               | 2.23 b                         | 1.3               | ...                            | ...               |
| 761                                    | CO       | 2.88 d   | 1.3               | ...                            | ...               | ...                            | ...               |
| 866                                    | WA       | 2.88 d   | 2.2               | ...                            | ...               | 2.70 e                         | 2.6               |
| 857                                    | WY       | 2.90 d   | 1.2               | 2.90 c                         | 2.0               | 2.47 e                         | 2.0               |
| 760                                    | CO       | 2.90 d   | 1.5               | 2.85 c                         | 2.2               | 2.46 e                         | 2.0               |
| 831                                    | WY       | 2.90 d   | 1.2               | 2.93 c                         | 2.2               | ...                            | ...               |
| 829                                    | WY       | 2.91 d   | 1.1               | 2.63 c                         | 1.6               | ...                            | ...               |
| 818                                    | MT       | 2.91 d   | 2.2               | ...                            | ...               | ...                            | ...               |
| 867                                    | ID       | 3.00 d   | 3.0               | ...                            | ...               | ...                            | ...               |
| 865                                    | OR       | 3.00 d   | 2.7               | ...                            | ...               | ...                            | ...               |
| 820                                    | MT       | 3.00 d   | 2.8               | 2.75 c                         | 2.6               | 2.72 e                         | 2.5               |
| 817                                    | MT       | 3.00 d   | 2.8               | ...                            | ...               | ...                            | ...               |
| Mean                                   |          | 2.3  | 1.2               | 2.0                            | 1.3               | 1.8                            | 1.5               |

<sup>x</sup> Rating basis: 1 = 0 to 3 lesions per needle; 2 = 4 to 10 lesions; and 3 = >10 lesions on needles. The ... indicates the seed source is not in the plantation.

<sup>w</sup> Sources arrayed according to their degree of infection of second-year needles in the Missouri planting.

<sup>y</sup> Number of trees of each source evaluated: Missouri 32, Nebraska 60, and Oklahoma 60.

<sup>z</sup> Means with the same letter within a planting are within the same cluster as determined by cluster analysis methods.

them were highly susceptible. All other sources in the study were of the variety *scopulorum*. The eight sources in the "Southern Rockies" cluster are of the "Southern Plateau" race of variety *scopulorum* (12); five of these sources were highly resistant, and three were moderately resistant. The rest of the sources of variety *scopulorum* were separated into five clusters. The "Central Montana," "Central Rockies," and "Colorado Plains" clusters contained some highly susceptible sources, but there were no highly resistant or moderately resistant sources. In contrast, among the 29 sources in the "High Plains" and "East—Low Elevation" clusters, 10 were moderately resistant and one was highly resistant, but none was highly susceptible.

This study identified several sources of ponderosa pine that are highly resistant to *D. pini*; these sources can be considered for seed collection for production of ponderosa pine seedlings in the Great Plains. Selection of resistant sources for seed collection can be improved by utilizing the data recently reported by Read (13). For instance, the highly resistant source 720 performed exceptionally well in nearly all of the 17 plantings. In contrast, the highly resistant sources from the Southwest grew well only in Nebraska and in the southernmost plantings (Missouri and Oklahoma).

The number of areas for seed collection also could be broadened to include some moderately resistant sources, because the impact of *D. pini* on moderately resistant sources having low infection of first-year needles is slight. On such trees, the newly developed

needles are not seriously infected until the second year. Therefore, in the central United States, the trees will receive the benefit of these needles for 2 yr, because symptoms do not develop until late (September and October) in the second year. Thus, a moderately resistant source, such as source 721 which performed well in most of the 17 plantings, could be utilized.

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