Variation in Resistance of Trembling Aspen to Hypoxylon mammatum Identified by Inoculating Naturally Occurring Clones

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ABSTRACT

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Inoculation during 1974 of natural clones of *Populus tremuloides* in the field with single-ascospore isolates of *Hypoxylon mammatum* produced significant interclonal differences in length of cankers after 70 days. More and larger cankers were produced from June inoculations than from those in July or August. The two most pathogenic isolates were selected and used during 1975 to test for differences in susceptibility among 100 clones of *P. tremuloides* and among 13 clones of *P. grandidentata,* occurring in 12 geographic areas of Michigan. Clones of *P. tremuloides* in northern areas had significantly shorter cankers than those in southern areas. Significant interclonal variability in canker development occurred in 10 of 12 areas. The amount of natural infection in each clone was not correlated with the length of artifically-induced cankers. *Populus grandidentata* became infected with *H. mammatum* after inoculation, but some clones showed resistance by profuse callus production.

Additional key words: hypoxylon canker, tree improvement, selection.

Aspen and other poplars are increasingly important raw materials in wood fiber industries. Quaking aspen (*Populus tremuloides* Michx.) provides 45% by volume of paper and boxboard fiber in the Lake States (5), and is used to a large extent in Canada (10). Hypoxylon canker, caused by *Hypoxylon mammatum* (Wahlenb.) Miller (syn. *H. pruinatum*), is a limiting factor in growth of aspen (1, 12). Genetic improvement of the species may be possible, especially for use in stands with rotation periods of 10-20 yr (4, 7), and such improvement could include resistance to Hypoxylon canker (13). Bigtooth aspen (*P. grandidentata* Michx.) is resistant to natural infection by the fungus. Intraspecific crosses of quaking aspen were infected more readily than were crosses with bigtooth aspen, or intraspecific crosses among bigtooth aspen alone (13).

The clonal growth of aspen in nature was described by Barnes (3). Copony and Barnes (6) indicated that clones differ in amounts of natural infection, but the meaning of this variability in terms of genetic resistance is not clear. We tested inoculation with living fungus mycelium as a possible technique for screening clones in situ for resistance to *H. mammatum,* and attempts were made to correlate the size of the resulting cankers with amounts of natural infection in the clones.

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MATERIALS AND METHODS

Inoculum.—Single-ascospore isolates of *Hypoxylon mammatum* were obtained from perithecial stromata on a naturally infected tree in Ingham County, Michigan. Eight spores were removed from a single ascus in serial order, and cultured on 2% malt extract agar. Nine additional single-spore isolates having known levels of pathogenicity (9) were obtained from New York State. The individual isolates demonstrated wide differences in morphology and pathogenicity. All cultures were maintained on 2% malt extract agar.

Moist sterile wheat grain was infested with mycelium of *H. mammatum* and used as inoculum after 3 wk of incubation. Preliminary tests had indicated that infested wheat grains were more effective as inoculum than were plugs from agar cultures.

Inoculations in 1974.—Four gynoecious clones of *P. tremuloides* near East Lansing, Michigan, were selected on the basis of root continuity between ramets, homogeneity of leaf morphology, and fall coloration (3). Two of the clones were growing on well-drained sites, and two on poorly drained sites.

During June, July, and August, a total of 39 trees on the periphery of each clone, 4-10 cm DBH, were inoculated with 12 *H. mammatum* isolates. Thirteen trees were inoculated each month with 12 *H. mammatum* isolates. Each tree was inoculated with four isolates (or three isolates plus a control on four small branches 6-10

cm from the main stem and about 2 m above ground. A 4-mm diameter wound was cut through the bark which exposed the xylem, and the bark plug was removed. Each wound was inoculated with a single infested wheat grain. Control wounds received a moist, sterile wheat grain. Wounds were covered with masking tape 2 cm wide. Isolates were grouped in an incomplete block design, with trees used a blocks. Care was taken to distribute the four replicate inoculations evenly around the somewhat circular area occupied by each clone.

Canker lengths were measured 70 days after inoculation, and data were subjected to analysis of variance with canker length as the dependent variable.

Inoculations in 1975.—One hundred natural clones of *P. tremuloides,* occurring in 12 geographic areas of Michigan (Fig. 1) and 13 clones of *P. grandidentata* in seven of these areas, were inoculated as described above in late April through early May, except that wounds were covered with 3-cm-wide Parafilm M (American Can Co. Greenwich, CT 06830). Clones were inoculated from south to north, coincident with spring leaf emergence, to reduce variability caused by differing phenology of the plants. Two isolates with high virulence and one isolate with low virulence in 1974 tests, and one control, were used as treatments on four separate branches of four replicate trees in each clone. Cankers were measured 70 days after inoculation.

Survey of natural infection.—The number of naturally-infected trees 4 cm DBH or greater in each of the clones inoculated in 1975 was counted when cankers were measured. A tree was termed infected if one or more cankers on the main stem were visible from the ground. All trees in most clones were examined. In clones with more than 100 trees, a transect 4 m wide was made through the center of each clone so that the ortet was included. Dead trees were counted as infected if *H. mammatum* stromal masses were visible.

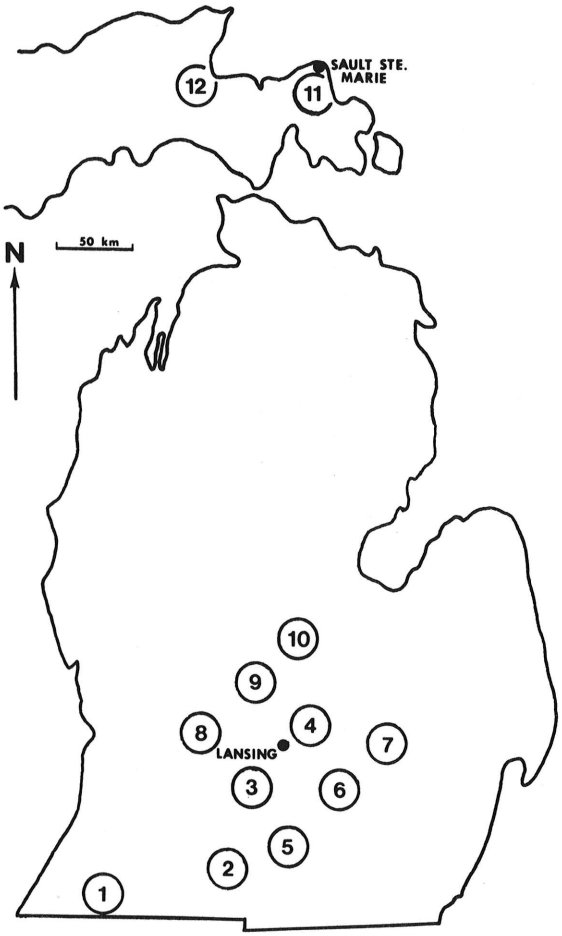

Fig. 1. Locations of 12 geographical areas wherein clones of *Populus tremuloides* and *P. grandidentata* were studied during 1975. Each area is approximately 30 km in diameter. Seven to 10 natural clones were randomly selected within each area, and inoculated with three isolates of *Hypoxylon mammatum.*

RESULTS

Inoculations in 1974.—Cankers became visible beyond the masking tape covers in about 6 wk, and extended rapidly in basal and apical directions. By 10 wk after inoculation cankered bark was wrinkled and cracked, with a yellow-orange mottle surface, typical of naturally occurring Hypoxylon cankers. Excision of outer bark exposed blackened tissue beneath the surface including the xylem near the inoculation wound. Some branches were girdled by necrotic tissue. The lengths of cankers were measured from apical to basal ends of blackened tissue after removal of outer bark.

Four of the 12 isolates were of low virulence and produced short cankers (21 mm or less) on 0-8% of inoculated branches. Five isolates produced cankers on trees inoculated in all three months, but three isolates produced cankers only in June and July. The fewest and smallest cankers resulted from inoculations made in August. Only six of 12 isolates produced cankers on all four clones during June and July. Statistical analysis was restricted to data obtained with these six isolates, from inoculations made during June and July.

Variability in canker length was influenced by month of inoculation, clone, and isolate of *H. mammatum* (Table 1). The longest cankers resulted from June inoculations.

Additional partitioning of the variance identified isolates that produced the greatest clone effects (Table 1). Isolates A and B produced the most (41 and 44 cankers, respectively, on 48 total inoculated branches) and the longest cankers. The isolate with the lowest pathogenicity caused one short canker on 48 inoculated branches. This isolate (605-6) had caused few cankers in previous tests (9). Isolates A and B and 605-6 were selected as having the highest and lowest pathogenicity, and were used in the 1975 tests.

Inoculations in 1975.—Cankers appeared sooner in 1975 than in 1974, extending beyond the paraffin film covers after about 4 wk. Cankers developed on 94.5% and 96.1% of 492 and 491 Branches of *P. tremuloides* inoculated with isolates A and B, respectively. Cankers ranged from 12 to 114 mm in length 70 days after inoculation. Isolate 605-6 caused 13 cankers, while

branches with control wounds produced four typical Hypoxylon cankers on *P. tremuloides*. These corresponded to 2.3% and 0.8% of inoculated branches, respectively.

Lengths of cankers produced by the two pathogenic isolates were subjected to analysis of variance. On *P. tremuloides,* canker length was influenced by geographic localities, clone, isolate of *H. mammatum,* and the interaction of isolate and locality (Table 2). Clones in northern areas (Fig. 1, areas 11 and 12) had significantly

TABLE 1. Analysis of variance of canker length caused by inoculation of four *Populus tremuloides* clones with six isolates of *Hypoxylon mammatum* during June and July, 1974

Effect	Degrees of freedom	Mean square	F significant at $P =$
Month	1	3,345.19	0.005
Isolate	5	2,814.45	0.001
Clone	3	5,356.45	0.001
Clone within isolate A	3	1,851.96	0.005
Clone within isolate B	3	1,196.56	0.035
Clone within isolate C	3	1,160.62	0.039
Clone within isolate D	3	1,138.59	0.042
Clone within isolate E	3	925.80	0.081
Clone within isolate F	3	869.10	0.097
Clone × Month	3	638.10	0.196
Isolate × Month	5	135.48	0.890
Isolate × Clone	15	296.35	0.737
Isolate × Clone × Month	15	189.99	0.473
Error	112	401.88[a]	

[a]Error mean square = s^2, where s = standard deviation of canker length within a clone; s = 20.0 mm.

TABLE 2. Analysis of variance of canker length caused by inoculation of 100 *Populus tremuloides* clones occurring in 12 geographic areas of Michigan. Clones were inoculated with two pathogenic isolates of *Hypoxylon mammatum* in April-May, 1975

Effect	Degrees of freedom	Mean square	F significant at $P =$
Areas	11	11,094.94	0.001
Clones (Areas)	88	1,396.82	0.001
Clones (Area 1)	5	1,774.77	0.001
Clones (Area 2)	7	1,878.73	0.001
Clones (Area 3)	9	618.23	0.111
Clones (Area 4)	6	852.44	0.041
Clones (Area 5)	7	2,663.17	0.001
Clones (Area 6)	9	1,766.05	0.001
Clones (Area 7)	8	1,429.14	0.001
Clones (Area 8)	7	1,631.89	0.001
Clones (Area 9)	5	1,946.26	0.001
Clones (Area 10)	9	938.01	0.011
Clones (Area 11)	9	486.41	0.254
Clones (Area 12)	7	1,467.53	0.001
Trees [Clones (Areas)] (Error)	346	384.09[a]	
Isolates	1	53,482.16	0.001
Isolates × Areas	11	718.49	0.016
Isolates × Clones (Areas)	88	359.23	0.313
Isolates × Trees [Clones (Areas)] (Error)	346	332.79	

[a]Error mean square = s^2, where s = standard deviation of canker length within a clone; s = 19.6 mm.

TABLE 3. Mean canker length (MCL) caused by inoculation of clones of *Populus tremuloides* with *Hypoxylon mammatum* during 1975, compared with levels of naturally-infected trees in the clones. Data are based on observations in area #5 of the experiment

Clone #	Number of cankers[a]	MCL			Natural infection	
		Isolate A	Isolate B	Isolate A + Isolate B[b]	Trees observed	Infected (%)
1	5	81	74	155 a	43	5
2	4	78	69	148 ab	54	14
3	5	61	62	123 abc	42	12
4	5	66	54	120 abc	51	20
5	5	65	51	116 bc	81	6
6	5	61	42	103 c	62	10
7	4	47	47	94 c	75	36
8	5	56	32	87 c	74	8

[a]Trees infected by only one of the two pathogenic *H. mammatum* isolates were excluded from the analysis.
[b]Means followed by a common letter are not significantly different ($P = .01$).

TABLE 4. Analysis of variance of lengths of cankers caused by inoculation of 13 *Populus grandidentata* clones with two isolates of *Hypoxylon mammatum*. Clones were located in seven geographic areas of Michigan, but were analyzed disregarding possible area effects

Effect	Degrees of freedom	Mean square	F significant at P less than:
Isolate A:			
Clone	12	12,518.99	0.01
Error	46	8,041.50	
Isolate B:			
Clone	12	69,860.70	0.01
Error	47	3,188.30	

shorter cankers than those in the south. Clones within 10 of the 12 areas differed ($P = 0.05$) from one another in canker length after 70 days. Isolate A usually produced larger cankers than isolate B (overall mean length of 58.5 mm and 45.7 mm, respectively). This difference varied among regions, however, as shown by the isolate × region interaction.

Amount of natural infection in clones of *P. tremuloides* ranged from 0% of the trees infected in 14 clones, to 58% in the clone with heaviest infection. Regression analyses were applied to 99 clones, with 20 to 100 trees in each. Percent of naturally infected trees in each clone was considered to be the dependent variable with each of three independent variables: (i) mean canker length (MCL) produced by isolate A; (ii) MCL produced by isolated B; and (iii) MCL produced by isolate A plus MCL produced by isolate B on the clone. Significant linear correlations between lengths of cankers resulting from inoculations and amount of natural infection were not observed in 11 of the 12 areas, with any of the three variables.

Data obtained from observations of clones in area 5 of the experiment are cited in Table 3 as an example of typical reaction to inoculation with pathogenic *H. mammatum* isolates. Although mean canker lengths among the eight clones varied significantly, percentages of trees in the naturally infected clones did not correlate with lengths of cankers caused by either isolate.

Branches of *P. grandidentata* inoculated with isolates A and B became infected, and produced cankers that were 13 to 99 mm long 70 days after inoculation. Isolates A and B produced cankers on 59 and 60 of 62 and 63 inoculated branches, respectively. Cankers in some clones were surrounded by large amounts of callus tissue. Analysis of variance of canker length among clones, disregarding possible geographic area effects, indicated significant clone differences in reaction to both highly virulent isolates (Table 4). At least 15 trees in each clone of *P. grandidentata* were examined, and only one naturally infected tree was found.

DISCUSSION

Previous work with *H. mammatum* demonstrated extreme variability in virulence of isolates (2, 9). Genetic variation in susceptibility of the host also was suggested among clones of *P. tremuloides* (6, 9), and among interspecific hybrids of *P. tremuloides* and *P. grandidentata* (13, 15). In nature, infection is most often associated with young lateral branches near the main stem (8, 11), or with insect galls on branches (11, 14). Therefore, lateral branches of young trees were inoculated in an attempt to measure the variation in susceptibility among clones.

The experiment in 1974 demonstrated that inoculations made in June produced more and larger cankers than later inoculations. In addition, use of *H. mammatum* isolates with high pathogenicity also contributed to production of cankers, with successful infection on 95-96% of inoculated branches in these tests. Single ascospore isolates may be more reliable for producing cankers than isolates from margins of natural cankers (16).

Differences among clones in length of cankers 70 days after inoculation were observed in 1974 and 1975. The observation of differences among geographical areas might indicate a macroclimatic control of canker size. However, the significant differences among clones within 10 of the 12 areas (Table 2) would indicate more clearly either microclimatic or genetic control of canker length. In addition, within-clone variability of canker length was small ($s = 20.0$ mm in 1974; $s = 19.6$ mm in 1975, Tables 1 and 2), in relation to interclonal differences. Hence,

highly significant differences ($P = 0.01$) between clones were indicated in most areas.

The meaning of this interclonal variability as it relates to resistance or susceptibility to *H. mammatum* could be questioned. Length of cankers following artificial inoculation generally was not correlated with amount of natural infection in the clones. However, infection in nature is subject to external variables which include inoculum density and environmental factors. For example, among the seven clones in area 9, 0% to 1% of the trees were naturally infected. This low level of infection may have been a result of a low geographic density of *P. tremuloides,* and therefore low amounts of inoculum. Artificial inoculation of these clones, however, produced cankers comparable in frequency and size to those observed in regions with greater incidence of natural infection. Amount of natural infection in a clone, therefore, may be a poor indicator of genetic potential for resistance or susceptibility in areas where aspen stands are far apart, or where the environment is variable.

Infection of *P. grandidentata* branches was observed after inoculation with *H. mammatum.* Rogers (15) also reported infection of *P. grandidentata* main stems by similar methods. Observations of large amounts of callus tissue around the diseased area in branches in some clones may be related to the low natural incidence of Hypoxylon canker among bigtooth aspen. The pathogen could be excluded from healthy tissue by this mechanism (15). Clones within this species also differed in canker length after 70 days, but vertification of this on a larger sample of clones is needed.

The *P. tremuloides* clones used in these tests were on "average" sites, that is, neither in standing water nor on particularly dry sites. However, microclimatic and nutritional differences among clones within geographic areas might affect lengths of cankers. Therefore, artificial inoculation tests may be most useful for preliminary identification of desirable host phenotypes in the field. Genotypes then could be screened intensively under a common environment after vegetative propagation. Since propagation is time-consuming, preliminary screening of clones in situ by inoculation or other means would be economically desirable.

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