GENETIC CONTROL OF SUSCEPTIBILITY TO HYPDXYLON MAMMATUM IN NATIVE ASPENS

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STUDIES IN NATURAL STANDS

Hypoxylon canker, caused by *Hypoxylon mammatum* (Wahl.) Mill. (syn. H. *pruinatum* (Slot.) Cke.) is one of the more serious diseases of trembling aspen *(Populus tremuloides* Michx.) but is rare on bigtooth aspen (P. *grandidentata* Michx.) (Graham et al., 1963). Burbee and Rogers (196)t) suggested that the reported cases of diseased P. *grandidentata* represent natural hybrids of bigtooth and trembling aspen mistakenly identified as P. *grandidentata.* Field surveys in the Lake States (Schmiege and Anderson, 1960), Michigan (Graham, et al., 1963), and Ontario (Dance and Lynn, 1965) indicate considerable variation in the incidence of hypoxylon disease in trembling aspen. This and the low incidence in bigtooth aspen suggest a genetic control of resistance to this fungus in the native aspens. Anderson (1964) suggested that genes for susceptibility in trembling aspen were dominant to those for resistance in bigtooth aspen.

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In 1968 the project herein reported was initiated to study the frequency and distribution of hypoxylon disease in the rather large but scattered population of P. tremuloides in northern New York State and to determine the nature of the genetic control of resistance and susceptibility to this pathogen in the two native aspen species.

MATERIALS AND METHODS

Twenty-five natural stands of P. tremuloides, rather uniformly distributed throughout and peripheral to the Adirondack Mountain region of northern New York State, served as the sample population for this disease survey. These stands, located in 1959 by Valentine (1961), were sufficiently large and old so that ten trees could be selected with a minimum distance between trees of 75 yards (to avoid duplication of clones) and a minimum diameter at breast height of seven inches. The ten "plot" trees were also free of any externally visible disease symptoms, frost cracks, or butt damage. Two hundred and twenty-three of the original plot trees were located by Dr. Manion in the winters of 1968-70, and the disease condition of these trees plus all trembling aspen within a one-chain radius of each plot tree was recorded. Results of this survey have been published (Manion and Valentine, 1971). Only the hypoxylon data will be summarized here.

Experiments for determining the genetic control of hypoxylon susceptibility in native aspens are based on large numbers of families of maternal and/or paternal half-sibling relationship, produced by controlled crosses. Diseased and nondiseased trees from the Adirondack population were crossed for the estimation of the heritability of this trait by the covariance of half-sib families. Eighteen families, six groups of three maternal half-sib families of 80 to 100 one-year-old trees each, were field planted in 1968 and first tested by artificial inoculations in the fall of 1969. Two families of 30 trees each, field planted as 1-0 stock in 1962 by Dr. Valentine for other studies, have been used to compare the pathogenicity of various sources of the pathogen as well as the reliability of the inoculation method. The parents of one family are both susceptible. Putative resistant trees (i.e. nondiseased trees from natural stands with high disease incidence so that "escapes" are less likely) served as parents for the other cross.

A large number of crosses of diseased and nondiseased trees of P. tremuloides, P. grandidentata, and the interspecific hybrid have been made since 1968. The parent trees, including three putative hybrids, are mostly from natural stands near Syracuse and Camden, New York. The remaining trees of both species and known F_1 interspecific hybrids are from The Harvard Forest, Petersham, Massachusetts, and were produced by controlled crosses by the late Dr. S. Pauley. Only a small proportion of the Massachusetts crosses have resulted in progeny groups of sufficient size for within and between family comparisons. (This is most likely due to the higher temperatures in the roof-top greenhouses of our new building rather than to natural incompatibilities, because we have had higher frequencies of cross failures, smaller seed yields per cross, and higher seedling mortality since occupying this facility in 1968.)

Twenty-four families representing 15 of the 16 possible intra- and inter-specific combinations of diseased and nondiseased trees of P. tremuloides and P. grandidentata were planted as 1-0 stock in 1969 in a randomized complete block design. The field plot has three replications, ten trees per family per replicate, and two border rows surrounding it. The initial inoculations were completed in July 1971. Groups of trees produced the past two years, mostly of limited family size, have been lined out at one-foot spacing.

The four inoculation methods that have been used are as follows: (1) direct insertion of ascospores into small wounds; (2) bark discs about 5 mm diameter, cut from the margins of cankers on living P. tremuloides trees at Heiberg Forest near Tully, New York, inserted into a small hole in the two-year-old portion of the stem, and covered with masking tape; (3) wood chip cultures produced by inoculating 2 mm cubes with a single ascospore and inserting the chip into a small hole in the stem as in the bark disc method; and ()4) barley grain cultures inoculated with mycelium from a single ascospore or a mass collection of ascospores from a fruiting body. The infected grain is inserted into a small hole in the stem as in the previously described methods. The sources of inoculum for the wood chip and grain cultures were from fruiting bodies collected from a canker on a living tree of each of the two aspen species and a known interspecific Fi hybrid at The Harvard Forest. Two ascospores were selected from a single ascus from each of the three fruiting bodies for a total of six single spore cultures. An additional three grain cultures were made using a mass collection of the spores. Inoculations were scored for survival and spread of the mycelium and for canker development two to three months later or, for fall inoculations, in the spring.

RESULTS AND DISCUSSION

Survey of Hypoxylon Incidence

Hypoxylon cankers occurred on 23 of the 223 plot trees located in 25 natural stands in northern New York, for a disease incidence of 10.3 percent among the older trees. This compares with a frequency of 9.2 percent for the more than 2100 trees scored which represent all size and age classes in these established stands. Of the remaining 200 plot trees, 59.2 percent were healthy, 8.1 percent dead of unknown causes, and 22.4 percent dead or dying due to other diseases, frost cracks, or a rough gall similar to crown gall (see Manion and Valentine, 1971, for complete results of this survey).

The incidence of hypoxylon-cankered trees among the 25 plots is shown in figure 1. Note that the 11 plots with 6 or more diseased trees among all trees scored (rated "high incidence") are peripheral to the Adirondack Mountains, except for the plot in Essex County (the second county down from the upper right corner) which is along the Cascade Lakes chain just east of Lake Placid, New York, in the heart of the "high peaks." Nine of the stands occur on abandoned farmlands and the other two, including the Cascade Lakes plot, on old burn sites. The six plots, in which none of the trees scored were cankered, are located on old burn areas in the central Adirondacks. The remaining eight plots, rated as "low incidence" with one to four diseased trees each, are scattered throughout the area with four on abandoned farmlands, three on old burn sites, and one whose past history is unknown.

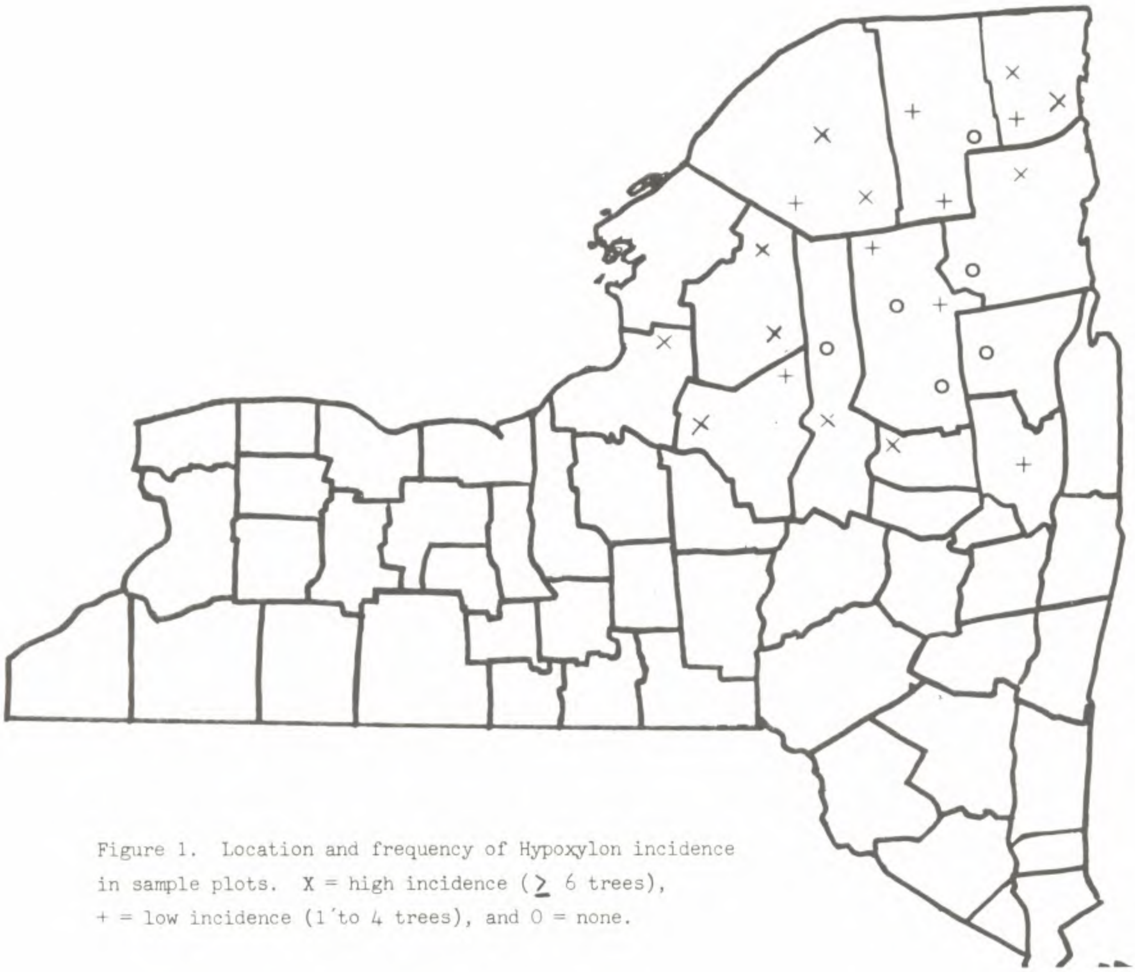

Figure 1. Location and frequency of Hypoxylon incidence in sample plots. X = high incidence ($\geq$ 6 trees), + = low incidence (1 to 4 trees), and O = none.

The data on hypoxylon incidence, tree age, and growth rate based on 215 plot trees, and plot elevation are summarized in table 1 by plots, grouped according to disease incidence among all trees scored, i.e., "High", "Low", and "None". There appears to be a negative relationship between disease incidence and both tree age and plot elevation, but a positive relationship with growth rate. The mean age of the 23 diseased plot trees is 35.5 years compared with a mean of 44.9 years for all plot trees. Seventeen of the 23 diseased trees are younger than the average age of all plot trees and, if each is compared with the other trees in their respective plots, 14 are younger than their plot mean age. If plots lacking hypoxylon are ignored, the mean ages of diseased versus all plot trees for the 11 "High incidence" plots are 35.4 and 38.7 years, respectively; and for the 8 "Low incidence" plots, 36.3 and 47.6 years, respectively. It is obvious that hypoxylon disease is more common in younger trees in northern New York State, which is in agreement with other studies (Anderson, 1964).

Table 1.--Frequency of hypoxylon in natural stands of *P. tremuloides*; relationship to stand elevation, tree age, and growth rate.

	Plot hypoxylon frequency			Total
	High[1]	Low	None	
Number of sample plots	11	8	6	25
Elevation, feet: Range	520-1960	900-1800	1600-2000	
Average	1190	1369	1753	
Number of plot trees	91	70	54	215
Mean age, years[2]	38.7	47.6	52.0	44.9
Mean growth (in./yr.)	.234	.202	.187	.211
Diseased plot trees:				
Number	20	3	0	23
Percent of total plot trees	22.0	4.3	--	10.7[3]
Mean age, years	35.4	36.3	--	35.5
No. younger than plot average	11	3	--	14
No. younger than mean age of all trees	15	2	--	17
Mean growth (in./yr.)	.255	.283	--	.258
Total trees scored, 2135 (all size and age classes)				
Number with Hypoxylon	199	19	0	218

[1] High incidence, ≥ 6 diseased trees; Low, 1-4 trees among all size and age classes.

[2] Age and growth rate based upon diameter cores taken at breast height in 1959.

[3] Differs from the 10.3 percent reported by Manion and Valentine (1971) as this is based upon the 215 trees for which age and growth rate could be determined, rather than the 223 trees they used.

The distribution of trees by age in the 12 plots in which disease occurred among plot trees is given in table 2. This clearly shows by the age distribution and average age of diseased trees compared with all plot trees that hypoxylon canker is more frequent in younger trees. The range in age of diseased trees, however, is surprisingly large, with the oldest, 59 years of age in 1959, hence close to 70 when the survey was made!

Table 2.--Age distribution of trees in twelve sample plots with one or more diseased plot trees.

Distribution by age	Number of trees	
	Hypoxylon	Total trees
19 or younger	1	3
20 - 29	6	11
30 - 39	7	31
40 - 49	7	37
50 - 59	2	19
60 - 69	-	3
Total trees	23	104
Mean age, years	35.5	40.9

The relationship of disease incidence with growth rate and plot elevation as shown in table 1 is probably not very meaningful since both reflect age differences. The growth rate, expressed as inches of radial increment per year, has not been adjusted for differences in age of tree so that any growth rate effect would be confounded with and probably obscured by an age effect. The negative relationship with elevation is also confounded, at least in part, with age. The lower elevation stands with high disease incidence are mostly on former farmlands, abandoned about the time of World War I, whereas, the high elevation stands with no disease are on sites of burns which occurred in the late nineteenth or early twentieth century. That age may be the important factor rather than elevation is supported by the fact that the exceptional, high elevation stand (1960 feet) along the Cascade Lakes with high disease incidence is among the younger stands, with a mean age of 36.1 years for the plot trees.

Disease incidence has been determined for 94 plot trees of known sex. Seven of the 39 females (18%) and 6 of the 55 males (11%) are diseased, indicating no obvious difference in susceptibility in the two sexes. However, the number of trees, particularly the diseased, may be too small to be a reliable sample.

The frequency of disease in the northern New York population represented by the 25 stands can be determined in several ways. The frequency among the 223 plot trees located in the 1968-70 survey is 10.3 percent and among all size and age classes, 9.2 percent (Manion and Valentine, 1971). Based upon all plot trees in the 11 "High" and 8 "Low incidence" plots, frequencies of 22 percent and 4.3 percent are obtained, respectively, with an average of 14.3 percent for the 161 plot trees in the 19 stands. If only the 12 plots are considered in which one or more plot trees are diseased, the respective frequencies are 27 percent, 10 percent, and an average of 22.1 percent.

A more meaningful disease incidence, however, may be that occurring in stands on abandoned farmlands. The distribution and frequency of genes for susceptibility and resistance to hypoxylon in these stands are probably more representative of the population than stands on old burns because of the origin of the trees. Trees on abandoned farmlands are more likely to be of seed origin, hence include a random sample of genes, whereas on old burns, they are probably of root sucker origin from old trees which survived selection due to this disease so that the genes present are not representative of the whole population. If this reasoning is sound, the best estimate of disease incidence in northern New York is 14 percent, based upon the 114 plot trees in fourteen stands occurring on abandoned farmlands. This, no doubt, underestimates the true frequency because in the 1959 survey when the plots were selected and trees sampled, though a random procedure was followed in selecting trees in each plot, the trees had to have a minimum diameter at breast height of seven inches and be free of external symptoms of disease. Disease frequency, determined in these various ways, is higher than reported for Minnesota (2 to 3 percent by Schmiege and Anderson, 1960) and Colorado (0.2 percent of living trees and 2 percent of the dead trees by Hinds, 1964), but in the same range or lower than in Maine (11 percent in closed stands and 21 percent in open stands by Schreiner, 1925), Michigan (22 percent, Graham et al., 1963), and Ontario (10 to 70 percent by Dance and Lynn, 1965).

Preliminary Report on Susceptibility of Families from Controlled Crosses

A summary of the parentage of the 62 families produced by controlled pollination for testing susceptibility to hypoxylon by artificial inoculations is given in Table 3. Each family consists of 25 or more full-siblings, one year or more in age. All but the families produced in 1971 have been field planted. Fifty-three trees, representing a large number of different natural stands throughout the Adirondack Mountains and near Syracuse, New York, served as parents, with 29 of these (17 female and 12 male) serving as the common parent for two or more families. The remaining 24 trees (2 female and 22 male) were each used for only one cross, but in each case, the other parent was crossed to two other trees. The total number of trees serving as male parents, 34, is shown by disease condition and by species across the top of the table. The 19 female parents are shown in the same way in the left column of the table. Note that two nondiseased hybrids are included among the female parents. The numbers in the body of the table indicate the total number of families for each combination of parents, e.g. the first group in the upper left indicates there are four families in which both parents are susceptible *tremuloides.* The number in the far right column in that same line, 21, indicates the total number of families with a susceptible *tremuloides* female parent.

Table 3.--Summary of families from controlled crosses for testing Hypoxylon susceptibility by comparisons of half-sibling families.

Female parents		Number of families for each combination of parents				
		Male parents				No. of families
		Susceptible		Nondiseased		
		tremu.	grand.	tremu.	grand.	
	No.	7	2	21	4	
Susceptible						
P. tremuloides	6	4	4	5	8	21
P. grandidentata	2	3	1	5	2	11
Nondiseased						
P. tremuloides	7	4	---	13	2	19
P. grandidentata	2	3	1	1	2	7
Hybrid	2	3	---	1	---	4
Total families		17	6	25	14	62

Sixty of the 62 families can be used for maternal half-sibling comparisons (excluded are two families in which the two female parents were used for only the one cross). These include two groups of six maternal half-sibling families (this can be symbolized as "1 x 6", with the first number conventionally the number of female parents and the second, the number of different male parents); two, 1 x 5 groups; one, 1 x 4; ten, 1 x 3; and two, 1 x 2. Many different combinations of parents, i.e. diseased and/or nondiseased of one or both species or the hybrid are represented in these groups.

The total number of families represented by each type of male parent is shown along the bottom of table 3. Forty of the 62 families can be used for paternal half-sibling comparisons. These include six groups of 4 x 1; four, 3 x 1; and two, 2 x 1.

Most of the crosses were planned for diallel comparisons, i.e. a given group of females would be crossed to the same group of males. Failure to obtain adequate numbers of seedlings and, in some cases, the cross itself, resulted in unbalanced groups of various combinations. Twenty-six of the families, however, can be used for diallel comparisons, which include one, 3 x 4; one, 3 x 3; and one, 2 x 3. Eighteen of the families represent an experimental design in which each of the six females is crossed to a random sample of three males. The 2)4 parent trees, all P. tremuloides selected at random from the Adirondack sample plots, include three susceptibles (one female and two males) and 21 nondiseased trees (five females and sixteen males).

The only results from artificial inoculations that can be reported at this time are for the 18 P. tremuloides families composed of six groups of three maternal half-sib families each. The first inoculations were made in the fall of 1969 by inserting ascospores into a stem wound on each of 80 to

100 trees per family. None resulted in a canker. These families were again inoculated in the summer of 1970 using bark discs as the inoculum for ten trees per family. The results are presented in table 4 along with natural infections that have occurred in these families and the results from a third inoculation in the fall of 1970 using the six single spore wood chip cultures for ten trees per family each.

Table 4.--Incidence of hypoxylon disease in eighteen *P. tremuloides* families; six groups of three maternal half-sibling families.

Common female parent	Random male parent	Number of diseased trees: Artificial inoculation: Bark disc 10 per family	Wood chip cultures, Spore source: *tremuloides* 10 per family	Wood chip cultures, Spore source: hybrid 10 per family	Natural infection	Total
CL101	ES111	1	---	---	---	1
	LE108*	---	---	---	---	0
	SL309	4	---	---	---	4
CL303*	ES201	2	1	3	---	6
	LE201	2	---	---	1	3
	SL111	2	1	1	---	4
ES212	FR106	1	---	---	---	1
	HE109	---	---	---	---	0
	OA102*	---	---	---	1	1
LE107	CL106	---	---	---	---	0
	CL306	1	---	---	1	2
	WA105	---	---	---	---	0
SL112	FR212	---	---	---	---	0
	FR311	1	---	---	---	1
	HE202	---	1	---	---	1
WA110	CL104	1	1	1	1	4
	FU109	3	1	1	---	5
	SA112	1	1	---	---	2
Total:						
Infected trees		19	6	6	4	35
No. inoculated		180	360	360	---	900
Mean freq. diseased trees		.106	.017	.017	---	.034[1]

* Known susceptible trees.

[1] Total mean frequency is based on the 31 infected trees from artificial inoculations.

Thirty-one successful infections were obtained, 19 from the bark discs and 12 from the wood chip inoculations. The latter group includes six from the inoculum derived from the P. tremuloides fruiting body, six from the hybrid, and none from the P. grandidentata source. Note that the mean frequency of infections from bark discs (10.6%) is more than six times that from wood chip inocula (1.7%). There is, however, a reasonably good concordance for the presence or the absence of disease by the two methods. No cankers were obtained from artificial inoculations in six families (one of these, ES212 x 0A102, has one natural infection), and cankers were obtained by both methods in five families, i.e. concordant results in 11 families. Results for only two of the remaining seven families, however, may really be discordant. One is family SL112 x HE202, in which a single canker was obtained from the wood chip inoculations. It is surprising that none of the bark discs resulted in infection. The other is family CL111 x SL309, in which four of the 10 bark disc inoculations resulted in cankers, but none from the 40 using wood chips. The results in the remaining five families cannot be considered discordant with any surety since only one canker was obtained by the bark disc method in each of four families and two in the fifth family. If these families represent some degree of resistance to the fungus, the lack of infections from the wood chip inoculations is not unexpected since it is only about one-sixth as effective in producing infections as the bark disc method.

The large variation in the frequency of diseased trees in the eighteen families indicates genetic differences in susceptibility to hypoxylon. The highest frequencies occur in the two groups of maternal half-sib families with CL303, which is a susceptible tree, and WA110 as the common female parents. Twenty-two of the 31 infected trees occur in these groups. One other family, CL111 x SL309, exhibits a high frequency from the bark disc inoculations, but, as previously mentioned, none as a result of the wood chip method. Five families have only one diseased tree each from artificial inoculations, and the remaining six families have none. Two families in this last group, CL111 x LE108 and ES212 x 0A102, have a known susceptible male parent, which suggests that susceptibility is recessive to resistance in trembling aspen. One tree in the latter family, however, is cankered due to a natural infection.

Conclusions on the genetic control of susceptibility and resistance can only be speculative at this time. It would appear that two or more loci control this trait and that resistance is either dominant, or, if dominance is lacking, due to additive gene effects.

The results from extensive inoculations made this summer, or in progress, will be available next spring and, hopefully, will clarify this problem. This past July, 21 of the 62 families represented in table 3 were inoculated using the bark disc method (these families include 15 of the 16 possible combinations of crosses of diseased and nondiseased trembling and bigtooth aspen). Also completed are the inoculations of two families of P. tremuloides using 10 different inocula (six single spore grain cultures, three mass spore grain cultures, and one bark disc) on each of the 30 trees in each family. Both parents of one family are known susceptibles, and of the other, nondiseased trees from stands with high incidence of hypoxylon. This experiment was designed to test the reliability of the grain culture method and to determine whether variability in virulence exists in various sources of the pathogen.

Inoculations in progress include several experimental plantings of P. _tremuloides_ produced by Dr. Valentine for heritability studies. Two 3 x 4 diallel experiments will permit both maternal and paternal half-sib comparisons for the 24 families of 30 trees each. Maternal half-sibling families that are being tested include three groups of 1 x 3 and six groups of 1 x 4; each of the 33 families is composed of 27 trees. The parents of these families represent a random sample from the Adirondack plots, so that the results of these tests will provide a good estimate of the heritability of this trait for the northern New York population of trembling aspen.

A major problem in our studies has been the low incidence of cankers from artificial inoculations. The bark disc method appears to be the most satisfactory. The difference in disease incidence from the bark disc and the wood chip methods of inoculations is most likely in the methods themselves, although differences in pathogen virulence cannot be ruled out. The absence of cankers from the inoculations with the single spore wood chip cultures derived from The Harvard Forest P. gr_andidentata_ fruiting body seems more than just coincidental even though canker incidence was low (12 cankers from 720 inoculations) for the other two types of wood chip inocula. It is more likely due to a lower viability of this source of the pathogen or a marked difference in virulence.

SUMMARY

A field survey of twenty-five stands of P. _tremuloides_ distributed throughout and adjoining the Adirondack Mountains in northern New York State shows 10.3 percent of the 223 older trees are infected by _Hypoxylon mammatum._ A frequency of 9.2 percent is found for trees of all size and age classes within a one-chain radius of each of the older trees. Disease incidence is higher on younger trees and in the younger stands on abandoned farmland peripheral to the Adirondacks; six older stands at high elevations in the central Adirondacks lacked the disease. The remaining eight stands, scattered throughout this region of the state, exhibit a low incidence of hypoxylon disease. Preliminary results from artificial inoculations suggest that resistance to hypoxylon in the Adirondack population of P. _tremuloides_ is controlled by two or more genes with resistance dominant or, if dominance is lacking, due to the cumulative effect of additive genes. The results of the testing of more than 2000 trees of known parentage by artificial inoculations will be available next spring. These experiments will provide reliable estimates of the heritability of this trait for the northern New York P. _tremuloides_ population and information on the genetic basis for the difference in susceptibility to hypoxylon in trembling and bigtooth aspen. Studies of the variation in virulence of the pathogen and methods for artificial inoculations are also in progress.

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